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Infant Metabolism

Proceedings of the World Health Organization's Seminars Held at Leyden and Stockholm in October November, 1950

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Introduction

In 1950 two international seminars on Infant Metabolism were held under the auspices of the World Health Organization in Leyden the Netherlands (October 15–30) and in Stockholm Sweden (November 1–15) at the invitation of the two countries Representatives from five neighboring countries (Belgium and France and Denmark Finland and Norway) together with a group of four from the United States and one Australian (a staff member of WHO) were invited to attend and participate in these seminars In all nine countries and twenty-one universities were represented

The project was the first of its kind to be undertaken by the World Health Organization Its purpose was to bring together a selected group of medical investigators with special interests in the broad field of infant metabolism so that scientific information might be exchanged on a research level among countries where such studies were currently in progress The small size of the separate panels constituting the seminars was conducive to informal presentations free discussion and critical evaluation—features which are not possible in larger scientific convocations The late Professor Evert Gorter served as a chairman of the seminar in Leyden Professor Arvid Wallgren in Stockholm

In broad terms the theme of the two seminars was similar and embraced current physiological biochemical nutritional and clinical problems in infant metabolism The topics reviewed in individual panels are presented as separate chapters in this volume

The meetings were conducted in English with at Leyden concurrent translation into French It is most regrettable indeed that the recording facilities in Leyden proved to be inadequate and that no satisfactory verbatim record of the proceedings was obtained The discussions that took place there under the able chairmanship of Professor Gorter could therefore not be included in this volume Fortunately much of what took place in Stockholm was a repetition of what had already occurred in Leyden and although this volume contains only the edited Stockholm proceedings it is in a sense a synthetic product of both the Leyden and Stockholm meetings since the formal presentations at many of the panels were essentially the same in both places

It should be added that the meetings in Leyden and in Stockholm not only provided an opportunity for an exchange of scientific information the record

of which is contained in this volume but they also served to establish intimate personal contacts and to cement friendly relations among the participating colleagues from the different countries

Thoughtful consideration was given to the idea that the original transactions be subjected to complete revision in order to bring them up to-date in view of the long interval which has elapsed between the meetings and this publication The decision was finally reached to present a verbatim report of the panel presentations and discussions so that it might serve as (1) an example of international exchange of current scientific information at a research level under the aegis of WHO and (2) a demonstration of pedagogical methodology for international distribution This volume is also published in the belief that it may serve as a comprehensive single reference source of data which are now scattered in numerous publications from many countries In this way it can conserve the time and energy of the scientist and clinician interested in this field of medicine

The participants in the seminars at Leyden and Stockholm are grateful to the World Health Organization for the opportunity provided them to attend the meetings The presence of Dr Pierre Dorolle Deputy Director General and of Dr Brock Chisholm at that time Director General of WHO at the opening sessions of the seminars at Leyden and Stockholm respectively stressed the importance attached to these meetings They are also indebted to the two chairmen of the seminars Professor Gorter and Professor Wallgren for their felicitous and instructive preparation and management of the conferences and to Professor E Grzegorzewski and Dr Erwin Kohn of the WHO staff for all the assistance given to them Finally the participants in the seminars owe gratitude to Dr and the late Mrs I Herbert Scheinberg whose painstaking editorial effort brought this volume to fruition

July 15 1955

S Z Levine M D
Chairman of Visiting Team

Contents

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Opening Session of the Stockholm Seminar and Official Addresses

Professor WALLGREN (Stockholm) As chairman of the World Health Organization s Seminar on Infant Metabolism I have the pleasure and privilege to extend to you a most sincere welcome I also express our gratitude for the honor conferred upon us by the presence of the representative of the Swedish government the Minister for the Department of the Interior Mr Eije Mossberg Thanks to the events of today we have also the honor to greet as our guest the Director General of the World Health Organization Dr Brock Chisholm I would also especially like to extend a hearty welcome to our foreign and Swedish colleagues lecturers at the seminar and members of the panels

The last three decades of this century have been marked by numerous international gatherings Politicians technicians writers scientists and members of other professions from different countries have met to discuss topics of common interest and to exchange experiences In the same way physicians have also assembled at recurrent international conferences But the greater the number of attending physicians the smaller the true benefit of the congress Therefore the custom has gradually developed of arranging congresses so as to gather together people especially interested in various circumscribed branches of medicine Congresses may for instance discuss only a certain type of diagnostic method prophylactic measure or therapeutic procedure such as electroencephalography BCG vaccination or lobotomy Even congresses such as this if they are open to everyone who wishes to attend have had a tendency to increase in size with the result that the benefits of personal contact have often been lost and the majority of persons attending have only been listeners who make no positive contribution to the solution of the problems under discussion and add nothing to the experiences related at the congress In order to increase further the profit of international medical scientific gatherings a new type of assembly has been introduced the symposium or the seminar in which only specially invited people have an opportunity to take part—people who have all made valuable contributions to the subject of discussion and are outstanding in their specialty and research work This is the type of scientific conference that is opened

today and its international character is stressed by the sponsorship of the World Health Organization and by the participation of specially invited scientists from six different countries

The subject chosen for discussion by the World Health Organization is metabolism in children For those who have little acquaintance with this theme it may seem somewhat excessive to devote fifteen days solely to discussion of metabolism in children and it may perhaps also be said that there are problems of greater importance than metabolism that ought to be solved Why precisely metabolism in children? There is hardly any other subject that is of such importance in the physiology and pathology of childhood The more we have learned about the laws and regulation of metabolism the more intensely we have realized the inadequacy of our knowledge and the complexity of the problems involved Since the advent of modern biochemistry pediatricians have been interested in and have contributed a great deal to the study of metabolism in children Some decades ago activity in this field of scientific work was such that it was said at least in Europe that leading pediatricians were more biochemists and laboratory men than clinicians This may still be true in some quarters but generally scientifically minded pediatricians today have succeeded in combining the laboratory and bedside study of patients

Our knowledge of metabolism in children has increased greatly and rapidly since the introduction of isotopes as a means of labeling and recognizing the different elements in organic and inorganic substances—salts foodstuffs and so forth The new knowledge of the function of endocrine glands that is rapidly accumulating has made it more evident that the hormones produced by these glands intervene very strongly in metabolism and act as important regulatory factors This is perhaps especially true about the newly discovered hormones ACTH and cortisone for which Kendall and Reichstein will receive the Nobel prize this year The latest results of studies in the United States and Europe and the rich new knowledge acquired in the post war years will be pooled and discussed at this Stockholm symposium The object is to exchange experience to give and receive new knowledge to promote enlightenment among pediatricians and biochemists in the important field of physiological and pathological metabolism in children and to inspire new scientific studies toward the preservation or restoration of the health of our children As the health of the people of the world greatly depends on the health of the children this enterprise is in conformity with the activity of the World Health Organization In the name of the children s physicians the pediatricians I want to extend our gratitude to the Director General of

the World Health Organization Dr Chisholm for arranging this seminar and on behalf of my country to express appreciation that Sweden has been cho en as its setting

Mr MOSSBERG (Stockholm) On behalf of the Swedish government I want to welcome to our country the highest official of the World Health Organization and his fellow workers We want you Dr Chisholm to know how much we appreciate your coming here and how glad we are to declare ourselves in entire agreement with your endeavors to give reality to the highest human ideals We understand quite well the necessity of concentrating the main efforts of the World Health Organization on the under developed countries at present We know that in addition to its head quarters at Geneva the World Health Organization has regional centers which make possible field work planned with regard to the very different conditions prevailing in various parts of the world Naturally we are especially pleased that the Organization also makes arrangements for the advancement of science by the exchange of experience and accomplishment between more advanced countries The course which is opening today is of this character

The study of pediatrics is rather old in our country In fact Professor Rosen called the father of pediatrics was a Swede In our time the basic sciences are exploring infant metabolism whence may come the solution of many problems in eradicating disease and promoting health Therefore we congratulate Dr Chisholm and his collaborators on the excellent idea of this course and we greet most heartily all the foreign scientists who have been willing to assemble here in Stockholm and participate in it

Developments during the last few years leave little place for undue optimism about the future of humanity One of the chief factors which give reason for hope is the World Health Organization We hope Dr Chisholm that you and all our guests will find in Sweden some achievements worth remembering which will inspire you with optimism when difficulties arise In fact there are few things more likely to unite men than the struggle against disease and the fight for the improvement of health It is in the interest of all nations and all groups to participate in this crusade the only aim of which is to alleviate the sufferings of men and in so doing to make the coming generation physically mentally and socially healthy

I have the honor to declare this seminar on infant metabolism the first program sponsored by the World Health Organization in Sweden open

Dr CHISHOLM (Geneva) Mr Minister Professor Wallgren ladies and gentlemen—

For the World Health Organization may I first most sincerely thank all of you who are participating in this meeting The World Health Organization is only beginning to develop toward its full range of responsibilities and this is one of the types of things which it hopes to develop to a greater extent in the future Among the responsibilities laid on the World Health Organization by the nations of the world are to promote and conduct research in the field of health and to promote cooperation among scientific and professional groups which contribute to the advancement of health It has not been clear to the World Health Organization or to the nations generally what the best methods of fulfilling these responsibilities would be It is believed that relatively small meetings like this one somewhat exhaustive in their approach detailed yet concerning themselves with the whole of the picture within a relatively narrow frame may result in a very valuable contribution to world knowledge in the field of health But such meetings are still experimental It will be of great assistance to the World Health Organization if any interested people participating in this experiment would send in criticisms or suggestions as to how this type of meeting could be improved on future occasions It is not to be expected that everything will be done in the best possible way at first However there is every confidence that to bring together hard working people with a wealth of observation experience and thought will be to everyone s advantage By this I mean not just to the advantage of the people who are fortunate enough to attend this particular meeting but to a much wider group of people throughout the world who may be expected to profit from these deliberations

The choice of the fields in which this kind of work should be undertaken is difficult It is a question of evaluating the stage of development at which this type of meeting would be valuable One hopes that there may be found a very considerable number of fields in which this may be the best approach There is no intention however that these meetings should take the place of large international conventions among technical people I think they should not and never will take that place But for clarifying issues within relatively narrow confines we believe they will be extremely valuable

May I say that I regret very much the circumstances in which we are meeting here in Sweden The death of the King of Sweden has given an atmosphere of sorrow not only to this country but far beyond its boundaries The respect that the King of Sweden has enjoyed throughout the world is generally acknowledged The validity of the constitutional monarchy has been shown in this country over a very long period Its social development dur-

ing that time has been recognized everywhere so that the sorrow felt by the people of this country is widely shared throughout the world It is clear that the motto adopted by the new King of Sweden is already the motto of the people of this country— Duty *First* is clearly a working principle in Sweden

The World Health Organization is very aware of the great advances that have been made in this country in the field of health The assistance that has been given to the work of the Organization by Sweden has been very great not only by the sending of excellent people to the deliberations of the World Health Organization but by providing opportunities for study in Sweden to fellows from other countries and by cooperating to the fullest pos sible extent with the World Health Organization in its attempt to fulfill its responsibilities I would only express my personal gratitude and that of the Organization to you Mr Minister and to all your officials for your very great helpfulness While it is not perhaps sound to pick out one particular person I must make some reference to Dr Hojer who in the deliberations of the World Health Organization has been a very real force for sanity for the common sense point of view and for nonpolitical attitudes on every possible occasion

The World Health Organization is in the hands of national delegations it has no separate existence beside what national delegations decide People who are sent by their countries to represent them in the World Health Assem bly and as members of the Executive Board are the people who decide the extent to which the World Health Organization will be able to fulfill its destiny The responsibility of every country to send the right kinds of people to the United Nations and all its specialized agencies is very great Only when the time comes when the people of the world think in terms of the United Nations and the specialized agencies as *we* rather than as *they* will the whole structure of the United Nations be able to do what it is set up to do The United Nations is founded on the hope—and it is no more than a hope at the present time—that there may be enough mature civilized world minded people found widely enough to be able to support this new kind of structure which in effect is going to become and is grad ually becoming a world organization It is quite clear that from now on a certain degree of world organization is quite essential to the security of the human race in fact to its very existence The degree to which the peoples of the countries of the world are able to support that attitude will decide the future of the human race We must remind ourselves that every time we bring together a group such as this we do something to draw lines across international boundaries to provide bonds of friendship and understanding

between people who live under quite different circumstances in different places This too, contributes something to the tying of the world together in mutual understanding and sympathy Much needs to be done and it requires much from a great many people Every person has a certain responsibility in his own field whether it be technical or political or any other Such cooperation is one of the pathways between countries The importance of this seminar lies in that area as well as in the technical results that may produce benefit to many people in many parts of the world

Infant Metabolism

CHAPTER I

Panel on Water and Electrolyte Metabolism

WATER AND ELECTROLYTE METABOLISM

Profes or BARNETT (New York) It will be my ... my very great pleasure to discuss factors controlling ... metabolism in young infants with emphasis on the ... mechanisms involved I pose this as a difficult probl... their inherent interest and their relation to the action of ... hormones these areas of physiologic study currently con... thought than almost any other in our laboratories in the ... from your journals I think this is no less true in your ... jects are difficult because new knowledge is accumulating ... acteristically this very fact contributes to the pleasure ... More important perhaps is the emergence of new con... tions being made As an example the kidney is con... end organ for many hormonal regulations The m... sodium potassium and inorganic phosphate is regul... *the posterior pituitary adrenal cortex and parathyroid* ... part through their effect on kidney tubules This rel... long been known However newer knowledge brings ... closer to hormonal regulation by suggesting that the k... endocrine organ The demonstration by Shorr and his ... material formed in the kidney and influencing blood ... normal as well as abnormal conditions provides one ... this suggestion I shall not pursue this subject now ... example of the important new concept which ...

tionships between different organ systems and the physiologic unity of the whole body making kidney physiologists out of endocrinologists and endocrinologists out of pediatricians interested in growth and development These concepts are based on observed facts and it is to these therefore that we must first look in our discussions

Recent advances in our knowledge of water and electrolyte metabolism in young infants have been made principally by extension of our knowledge in several related but rather well defined physiologic areas These might be

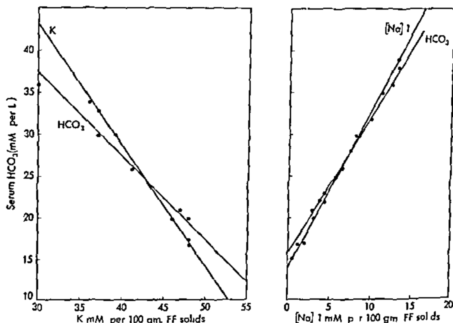


Fig 1 Relation of the concentration of bicarbonate in serum to the total potassium and intracellular sodium of rat muscle per 100 gm of fat free solids For meaning of straight lines see original source—Darrow D C Schwartz R Iannucci J F and Coville F *J Clin Investigation* 27 202 1948

described as (1) recognition of the transfer of electrolytes particularly of potassium and sodium between intra and extracellular fluid compartments (2) application of improved techniques for measuring the volume of body water compartments (3) recognition of the functional immaturity of the kidney in young infants and (4) the application of improved techniques for investigation of hormonal control of water and electrolyte distribution and excretion

The present status of our knowledge in these four areas provides the material for detailed discussion in some of the panels of the next two weeks

This afternoon I should like to describe very briefly examples of work in each of these fields and to indicate how they are related to the problems of water and electrolyte metabolism in young infants

That potassium and sodium are transferred between intra and extracellular fluids under a variety of conditions now appears to be well established Darrow has described an important reciprocal relationship between concentrations of extracellular bicarbonate and intracellular potassium which is shown in Figure 1

In experimental animals he has demonstrated that under certain well defined conditions if extracellular bicarbonate is increased by any means

ADULT

Plasma
Extracellular fluid
20%

Intracellular fluid
50

Solids
30

INFANT

Plasma

Extracellular fluid
50%

Intracellular fluid
30

Solids
20

Fig 2 A diagnostic representation of the composition of the body of an adult and of a premature infant weighing about 2000 gm (McCance R A. *Am J Med* 9 230 1950)

intracellular potassium decreases Conversely primary reduction of intracellular potassium leads to elevation of serum bicarbonate These relationships have important physiologic and clinical implications which will be discussed in a subsequent panel

Turning to the second point of measurement of body water compartments shown in Figure 2 taken from a recent review by McCance we see the classical concept of the volumes of these compartments and the differences between infants and adults in the relative sizes of these compartments

These diagrams demonstrate the well known facts that the total water content of the infant s body is proportionately greater than that of the adult s and as shown by Kerpel Fronius Gamble and others this difference is accounted for almost wholly by a proportionately greater volume of extracellular fluid Although the general principles of distribution of body water shown in this illustration are valid recent work indicates that quantitative corrections

need to be made If one uses the inulin space measured after a long period of equilibration as the best estimate of the volume of extracellular fluid the figure for this volume in the adult is probably closer to 15 per cent than to 20 per cent of body weight Using deuterium or antipyrine spaces as estimates of total body water brings this figure from 70 per cent down to

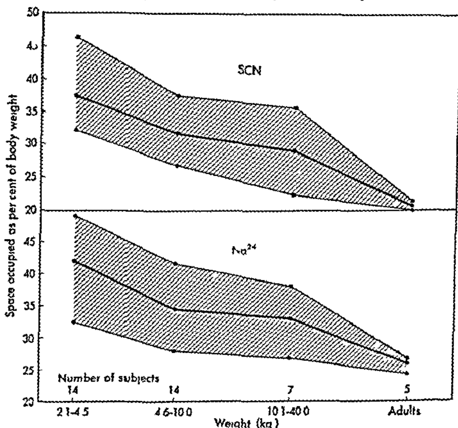


Fig 3 Decrease in SCN and Na^{24} spaces with increasing weight

50 to 55 per cent of body weight A study of deuterium and antipyrine spaces in infants and children has been made by Friis Hansen who fortunately is here today However to my knowledge extensive measurements of other body water compartments by newer methods have not yet been made in infants and children of different ages We can assume that the trend of changes in extracellular fluid volume with growth follows that observed with thiocyanate and radioactive sodium spaces shown in Figure 3

Here we see a marked decrease in these spaces with increasing body weight in subjects ranging from premature More accurate meas

urements of these body water compartments in infants are needed and are of more than physiologic interest Newer approaches to the problems of treatment of losses of water and electrolytes during dehydration in infants require more quantitative estimations of the volumes of body water compart ments as influenced by growth The introduction many years ago of methods for administering arbitrary amounts of water glucose and sodium chloride to dehydrated infants has without question been a great advance in treat ment and has strikingly reduced the mortality in such diseases as severe diarrhea of infants The success of such qualitative treatment depended to no small extent upon the ability of hormonal and renal mechanisms to retain the water and electrolytes needed by the body and to excrete those which may have been and often were given in excess In general terms we now

FIGURE 4
Kidney Function in Premature Infants*

	CLEARANCES				TM/PAH	C_{IN}/C_{PAH}
	INULIN	MANNITOL	UREA	PAH		
Mean Value ml /min	4 65 ±0 90	3 96 ±0 86	2 44 ±0 68	14 55	1 26	0 35
Number of Observations	111	132	229	23	25	19
Number of Subjects	13	23	22	7	7	6

Age 3– 8 days wt 2040–2400 gm S A 0 163–0 178 m

know that kidney function in very young infants is not fully developed Measurements of discrete kidney functions in premature infants are shown in Figure 4 which gives mean values for clearances of inulin mannitol urea and PAH and for maximum tubular excretion of para aminohippurate

Corrected for surface area these values range only from 16 (Tm) to 37 per cent of expected normal adult values One effect of this renal immaturity on water and electrolyte excretion is shown in Figure 5 taken from work of Dean and McCance in which the behavior of the kidney of an infant and of an adult was observed under the stress of an osmotic diuresis during hydropenia

The scale for the infant is one tenth that of the scale for the adult When sodium chloride was used to raise the osmotic pressure of the body fluids diuresis in the infant was small and prolonged in contrast to the intense diuresis observed in the adult The effects of renal immaturity require much more investigation but it seems clear from present knowledge that this factor

must be included in our consideration of water and electrolyte metabolism in young infants

Although the pattern of development of endocrine functions with growth is only beginning to be studied in detail hormonal regulation of water and electrolyte metabolism also may be incompletely developed in very young

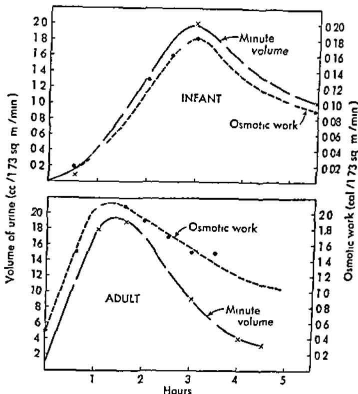


Fig 5 *Changes in the osmotic work of the kidney and in the minute volume of the urine after the administration of hypertonic saline to an infant and an adult* Note that the scale for the infant s osmotic work and minute volume is one tenth that of the scale for the adult (D an R F A and McCance R A Renal Responses of Infants and Adults to Administration of Hypertonic Solutions of Sodium Chloride and Urea, *J Physiol* 109 86 1949 Cambridge University Press)

infants and is consequently of equal or greater importance Recently applied techniques permit more quantitative approaches to the study of hormonal regulation of water and electrolyte metabolism an example of which is shown in Figure 6 taken from work of Levitt and Gaudino

Here some of the effects of total adrenalectomy in a dog are shown In these observations total body water represented by the first series of bar diagrams on the top of the figure was measured with *deuterium* The next

line represents changes in intracellular water which were calculated by the difference between total and extracellular water measured with inulin and shown on the third line Changes in inulin clearances and in maximal tubular excretion of para aminohippurate are shown in the last two series of diagrams Treatment and time in days after adrenalectomy are shown at the bottom

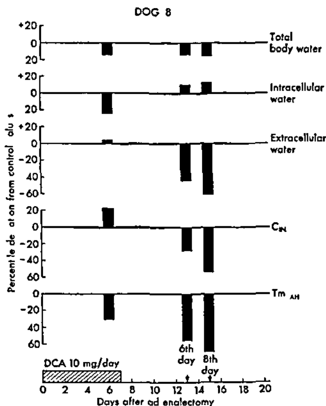


Fig 6 Effect of total adrenalectomy in the dog Arrows indicate days on which experiments were performed after cessation of DCA injections (Gaud no M and Levitt M *J Cl n I vest g tion* 28 1493 1949)

All values are expressed in terms of percentile deviation from control values The changes from preoperative values shown while the dog was receiving desoxycorticosterone after adrenalectomy were similar to those observed in normal dogs treated with desoxycorticosterone Intracellular water was decreased, and extracellular water increased along with an increase in inulin clearance Examinations made 6 to 8 days after desoxycorticosterone was discontinued show changes which become more conspicuous as the degree of adrenal insufficiency increases Changes in distribution of body water

are the most prominent features These changes show a marked decrease in the volume of extracellular fluid accompanied by an actual increase in intra cellular fluid with almost no change in total body water At this point intra cellular water accounted for 81 per cent and extracellular water for only 19 per cent of total body water *At this same time values for plasma volume* not shown here corresponded to one half of the extracellular volume In this study the volume of distribution of radioactive sodium and potassium was measured and it together with known concentrations of sodium and potas sium in serum permitted calculations of changes in intracellular concentra tions of these electrolytes Although this type of observation is difficult and has not been made extensively in young infants such techniques as these must ultimately be applied to investigations of the effect of immaturity growth and disease on hormonal control of water and electrolyte metabolism

Investigations in these four areas are of great physiologic interest but their final importance extends as it should to clinical application in the treatment of sick infants It is our strong conviction that further improvement in our treatment of diseases associated with abnormal losses or retention of water and electrolytes must stem from more quantitative treatment of individual infants This attitude requires among other things extension of our knowl edge of the influence of growth on the factors I have mentioned

TECHNIQUES FOR ESTIMATING BODY FLUID COMPARTMENTS

Professor BARNETT (New York) There is a higher proportion of extracellular fluid in the infant with a smaller proportion of intracellular fluid and solids (See Fig 2) As already mentioned the accurate measure ment of and the influence of growth on these spaces becomes of increasing importance with our attempts to define more quantitatively the fluid require ments of dehydrated infants of different ages and states of physiologic maturity

With our present methods our knowledge concerning body water compart ments would seem to indicate that intracellular fluid maintains a fairly constant proportion throughout the period of growth and that the decrease in total body water in per cent of body *weight which occurs from birth to adulthood* results more exclusively from a proportionate decrease in extracellular fluid

The present methods of measuring these body water compartments are by *no means entirely satisfactory* I should like to indicate a representative calculation of changes in the three major body water compartments arrived at by using the method so extensively explored by Darrow and others I am doing so more for the purpose of its evaluation in view of our recent knowledge

than to recommend it as an absolute measure of changes in body fluid compartments I think it is used frequently enough to make it worth while going through a sample calculation

If we use short balance periods of say one to three days with an infant whose initial body weight is 10 kg and make the first assumption that extracellular fluid represents 25 per cent of body weight we obtain an initial extracellular fluid volume of 2500 ml By determining the chloride concentration in the serum assumed here to be 100 mEq per liter and by making what I believe is a valid assumption that the chloride concentration in serum represents that in other extracellular fluid we can then calculate the total amount of chloride present in the body as being 250 mEq By measuring intake and output during this period we can determine the chloride balance which here I have assumed to be plus 50 mEq The final amount of chloride then becomes 250 plus the positive balance of 50 or 300 mEq of chloride If the chloride concentration of serum is at this time again found to be 100 mEq per liter we can then calculate that the final extracellular fluid volume is 3000 ml assuming that chloride is exclusively extracellular and has remained so throughout the period If the body weight has increased by 3 kg which can be measured and if it is assumed that the change in body weight is due entirely to change in body water which is a fair assumption in short term observation we can then calculate that there was a decrease in intracellular fluid of 200 ml

By using such a technique changes in the concentration of various electrolytes in intracellular fluid can also be calculated I think however we must point out errors inherent in this method Perhaps the most serious one is the assumption that chloride is entirely extracellular because there is very good evidence to the contrary The second one is the assumption of the initial extracellular fluid volume However changes in this figure make very small changes in the final calculation Still another defect in this method is failure to include in the balance estimations the chloride that is lost in sweat. Darrow has made strenuous efforts to estimate and even to measure this loss but I think it presents a real difficulty in the application of this method.

With these limitations I think interest increases in more quantitative and direct measurements of body fluid compartments and concentrations of electrolytes in the various body fluids The currently investigated methods are modifications of the dilution technique which as pointed out by Levitt and Gaudino depends on the use of a substance usually foreign to the organism which satisfies the primary criterion of uniform and extensive distribution throughout the space being measured If this is true the volume of the compartment being measured whether it is total body water

extracellular fluid is equal to the amount of this substance in the compartment divided by the concentration in the compartment As they have stated ideally such a substance should also fulfill the following conditions (1) there should be rapid and uniform distribution of the substance in the compartment that it is supposed to measure (2) there should be no formation or destruction of the substance in the organism (3) there should be no influence of the substance on the volume of body water compartments or on membrane permeability (4) there should be slow elimination of such a substance from the body (5) there should be no toxicity and (6) and very important the

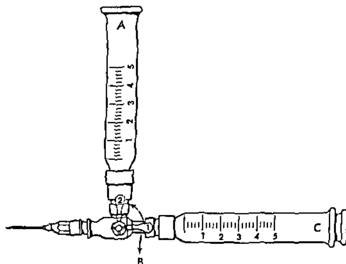


Fig 7 Apparatus used in a simple quantitative method for intravenous injection of small volumes of fluid See text for details

determination of the substance should be practical and accurate This method has been employed most extensively in the past for measuring thiocyanate spaces although it was realized in the very first paper on this subject that thiocyanate does not have an exclusively extracellular distribution and it was suggested as a measure of changes in rather than an absolute measurement of extracellular fluid Since this time as so frequently happens the method has been improperly interpreted as an absolute measure Not because I think either thiocyanate or sodium[2] space represents good measurements of extracellular fluid but rather because in young infants I am not at the present time certain that we have a better relative estimate I should like to show the changes in thiocyanate and sodium spaces with growth

First I should like very briefly to show the technique we employ for the quantitative injection of small volumes of fluid in young infants Instead of

using a calibrated syringe we use two syringes connected by a 3 way stop cock as shown in Figure 7

Volumes of fluid to be injected are pipetted accurately into syringe *A* and then withdrawn into *C* and injected after which *A* is washed out with small

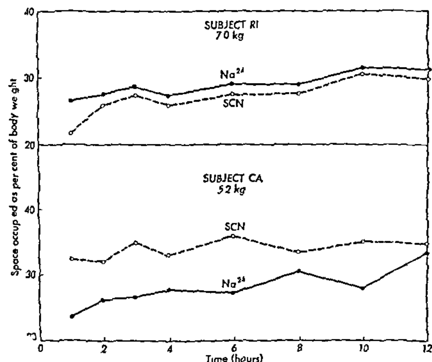


Fig 8 Changes in Na and SCN spaces over 12 hours in infants (Fellers F X Barnett H L Hare K and M Namara H Change in Thiocyanate and Sodium Spaces during Growth *Pediatrics* 3 6_3 1949 Charles C Thomas Publisher)

amounts of saline This provides the very distinct advantage in small infants (as well as in small animals) that several substances can be given in a single injection

Attempting to define the influence of growth on extracellular fluid volume we measured thiocyanate sodium inulin and mannitol spaces after single injections Figure 8 represents changes in space occupied as per cent of body weight of sodium[2] and thiocyanate over a period of 12 hours in two infants weighing 7 and 5 2 kg respectively These two substances reach a rapid early equilibrium but the space occupied by sodium[2] slowly increases over a period of 10 to 12 hours By tissue analyses in experimental animals

this can be demonstrated as being due to the fact that sodium^{4} diffuses and reaches equilibrium in extracellular fluid and in some intracellular fluids very promptly within perhaps a matter of 2 to 3 hours after which there is a further slower distribution of the substance which probably represents equilibrium being reached with sodium in bone As shown by Mannery Kaltreider and Bale this slower distribution represents a figure of about 10 per cent over the initial value

That these spaces decrease with growth was shown in two subjects who were observed serially at the ages of two and ten weeks and two and five or six months

Group data on a number of subjects revealed values shown in Figure 3 (p 4) where middle solid lines are the means and the striped areas represent the range of values observed It can be seen that both thiocyanate and sodium^{4} spaces underwent a parallel change when they were measured in subjects of different weights ranging from premature infants weighing 2 kg to adult subjects I would point out two things in this figure One is the rapid decrease in these spaces from birth until the weight of about 10 kg after which a plateau seems to be reached with a further decrease at adolescence I do not know of any confirmation of this change at adolescence and I would be interested to know if anyone has data concerning this point

The second thing I would point out is that in interpreting these data we reasoned that since thiocyanate and sodium^{2} which were very different electrolytes showed the same change in distribution this probably represented a change in the volume occupied rather than a change in the permeability of membranes to both electrolytes In other words if this decreased space were due to a change in the distribution of thiocyanate between intracellular and extracellular fluid one would not expect sodium to follow it so exactly whereas if it were actually due to a decrease in a space one would expect the two to show a parallel change This however is not a very valid argument because it is conceivable that membrane permeability or whatever determines the intracellular distribution of these two ions may be changing at different periods of growth

An attempt was made to fortify this argument further by measuring inulin and mannitol spaces after a single injection However the results were discouraging After single injections of mannitol inulin sodium^{4} and thiocyanate into two adult subjects we can see in Figure 9 the same general changes of the sodium space rising above the thiocyanate space as it enters bone The inulin and mannitol spaces continue to increase with time and never reach a plateau In the present instance these substances reach a volume of distribution which is completely unreasonable as a measure of extracellular fluid

The fact that these values for inulin and mannitol do not represent diffusion of inulin and mannitol into intracellular fluid is shown in Figure 10 illustrating *the same continuing increase in the size of the spaces in an intact dog given* a single injection of three of the substances When the renal pedicle is ligated and the single injections given it can be seen that the inulin space

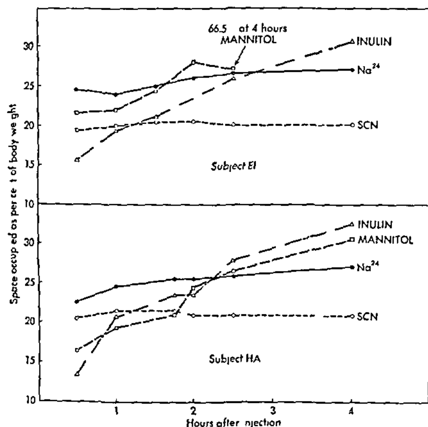


Fig 9 Relation of Na^{24} SCN inulin and mannitol spaces to time in two adult subjects

does reach a plateau and that the mannitol space shows a slight increase which can be explained by the known metabolism of small amounts of mannitol

The error produced by the use of the single injection as shown in this figure can probably be explained on a technical basis During the period of equilibration when inulin and mannitol are excreted very rapidly a very small error in the determination of the amount of mannitol lost from the body during

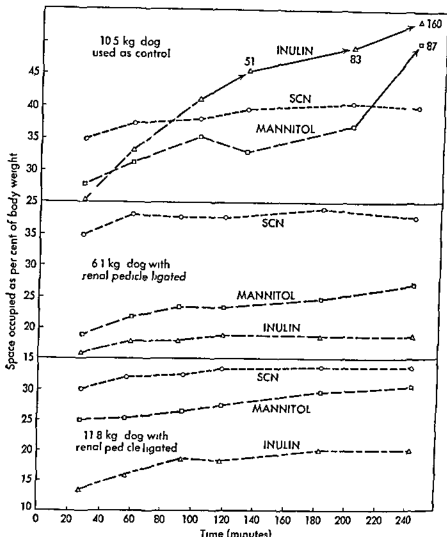


Fig 10 Relation of SCN mannitol and inulin spaces in dogs with ligated renal pedicles (Fellers F X Barnett H L Hare K and McNamara H Change in Thiocyanate and Sodium-4 Spaces during Growth" *Pediatrics* 3 6?6 1949 Charles C Thomas Publisher)

the first half hour (when the concentration is very high) can produce an error of 100 per cent in the calculation of the amount of inulin and mannitol left in the body at the end of an hour after equilibration has been reached As a result at least in our experience the measurement of inulin and mannitol *spaces after a single injection has not proved satisfactory We can measure* sodium and thiocyana e spaces but we should like to be able to measure

extracellular fluid rather than the distribution of substances which are both extracellular and intracellular

In adults I think this problem is partially solved in a somewhat complicated manner by the measurement of inulin spaces after a long-continued infusion as introduced by Levitt Gaudino and Schwartz I would like to consider some of their results because I think that at the present time this technique permits the best measurement of extracellular fluid space On the other hand I am not certain that this technique is applicable to small infants and I think this poses our greatest problem at the present time in the measurement of these fluid spaces

As previously described we were unable to get a constant volume of distribution in intact subjects by giving a single injection of inulin measuring the amount excreted and calculating the amount left in the body by subtracting the amount excreted from the amount given However the amount left in the body can be determined by giving a slow infusion until equilibrium is reached and the blood level and rate of excretion are constant and then stopping the infusion and simultaneously drawing a blood sample and emptying the bladder The amount of inulin in the body at the time the blood sample is drawn and the concentration measured is determined by measuring the amount which is excreted over the period of the next 12 or 24 hours Using this technique Levitt and Gaudino were able to demonstrate a constant volume of distribution of inulin Evidence concerning the time at which equilibrium is reached is shown in Figure 11 The fall in plasma inulin concentration is recorded in the upper curve after a single injection in which the very rapid fall immediately following injection is due both to excretion and also to diffusion of inulin into other extracellular fluids The fact that the curves for the rate of fall of inulin in serum after infusions of 4 5 5 5 and 18 hours duration are superimposable is further evidence that after 4 5 hours inulin leaves the blood only by excretion through the kidneys and not by further distribution in other body fluids

In these observations calculation of delay time is necessary if the urine flow is less than 2 or 3 ml per minute In this case the delay time represents the time necessary for urine to pass from the glomeruli to the catheter at different urine flows At very low urine flows this can introduce an error as great as 8 per cent in the determination of inulin space by this method At rates of urine flow above 2 or 3 ml per minute the error is insignificant

I have spent considerable time on this because I think that one of the important matters for discussion is the problem of how we can measure extracellular fluids in small infants

I am afraid a continuous infusion given for 5 hours to a small infant would

change his extracellular fluid enough to render the value rather unreliable However it is not inconceivable that with mechanical pumps we could get an infusion which could flow constantly at a rate that is small enough to allow this to be done Katcher Levitt Sweet and Hodes have recently reported such measurements on young infants during recovery from diarrhea However values for normal infants are not yet available

By using inulin spaces so determined as a measure of extracellular fluid volume values of 19 4 per cent in dogs and 15 3 per cent in adult human subjects have been obtained

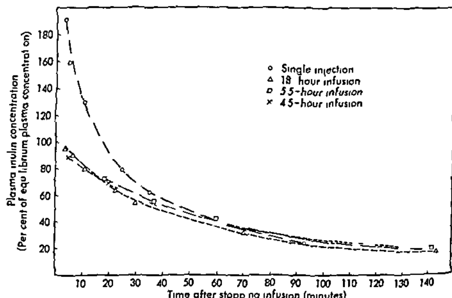


Fig 11 Concentration of inulin in plasma following a single injection and infusions of 4 5 5 5 and 18 hours (Schwartz I L Schachter D and Freinkel N *J Clin Investigation* 28 1119 1949)

If inulin spaces are assumed to measure extracellular fluid then approximately half of thiocyanate approximately a third of the sodium and approximately a fourth of the chloride are intracellular

I think before going on to the discussion of measurements of total body water we might stop and hear of any other experiences with the measurement of extracellular fluid in either young infants or other subjects

Dr FRIIS HANSEN (Copenhagen) I am of the same opinion as Dr Barnett that the relative decrease found in extracellular fluid during childhood is confined only to the extracellular space and that it is a real decrease not a false one based upon a change in permeability The water content in the cells is constant I believe I think that we have evidence of this if we

compare Dr Barnett s results where he found the extracellular space to be 40 per cent of the body weight with ours where we found 80 per cent total body water which by subtraction gives 40 per cent intracellular space in the newborn In adults we found 20 per cent extra and 40 per cent intracellular space Hastings while investigating histochemical changes during aging in rats also found that the intracellular water was practically constant from birth until senium and that if anything it increased a little in the last period whereas the extracellular space decreased

Chloride is found in high concentration in the gastric juice Do any of these methods measure water sodium and chloride in the gastrointestinal tract? We can expect fairly large changes in these volumes since in the adult the volume of the daily intestinal excretion is about 8 liters and at a given time it is possible to find 1 liter or even more in the gastrointestinal tract This is a high percentage of total extracellular fluid

Professor BARNETT (New York) I am very interested to hear that the result with deuterium spaces indicates a relative constancy of the proportionate value of intracellular fluid with growth

The question as to whether equilibrium is reached with water in the gastrointestinal tract has to my knowledge not been directly tested We have no such data and insofar as I know nothing has been published to indicate whether or not inulin reaches equilibrium with water in the gastrointestinal tract I think that there is some indirect evidence to suggest either that water in the intestine is excluded entirely and is fairly constant or that equilibrium with that water is attained during a period of 5 to 6 hours The former does not seem probable The other possibility that equilibrium is reached is suggested by the fact that the inulin spaces become so constant after equilibration is reached The values are also remarkably constant from one day to another in a given subject according to the data of Levitt and Gaudino Experimentally one could analyze in experimental animals intestinal water after different periods of infusion as has been done with ascitic and other fluids

More indirect data on this point will I think be forthcoming when this technique is more fully applied to disease conditions in which marked variations in the volume of gastrointestinal water can be expected During the first 12 hours or so after the onset of diarrhea in an infant evidence of very marked dehydration may be noted before any real increase in stool volume occurs and this has been interpreted as representing a large accumulation of water in the intestinal tract If at this time measurement of the inulin space shows it to be very greatly decreased without changes in total body weight it would amount to indirect evidence bearing on this problem

Dr FRIIS HANSEN (Copenhagen) We have tried to inject heavy water intravenously and have then introduced a stomach tube and withdrawn samples After five minutes deuterium is found in the gastric juice and after 10 minutes it is completely in equilibrium However we have not tried any of the extracellular substances

Dr USSING (Copenhagen) Does inulin diffuse through the brain barrier? The volume of the brain and the nervous system may be outside the volume which is accessible to inulin Normally even sodium takes a long time to diffuse through the brain barrier and a molecule of the size of inulin may take a very long time or may not even reach equilibrium in a reasonable time

Professor BARNETT (New York) I do not recall any direct observations on rate of equilibration with spinal fluid As judged from constancy of distribution after different periods of equilibration either inulin does reach equilibrium in this time or the difference is outside the quantitative limitations that can be demonstrated

Dr SJÖGREN (Stockholm) We have tried to measure extracellular fluid with mannitol according to the Schwartz method We give mannitol in continuous injection then after discontinuing the injection we measure the rate of disappearance of mannitol in the plasma We have tried to calculate the extracellular volume from the total clearance and the slope but have had difficulties It has been easy to obtain a constant level of mannitol but it has been impossible in many cases to obtain a straight line We think this may be due to the fact that the glomerular filtration rate changes and a change of only 10 to 15 per cent makes a big difference in the value of extracellular volume

Professor BARNETT (New York) We have not recently attempted to calculate extracellular fluid volume from the rate of disappearance of mannitol from blood A number of years ago we were interested in using the slope of the line representing the disappearance of inulin as a measure of glomerular filtration rate in young infants We were able to show a rough correlation between the slope of the line and actually determined clearance Calculating the volume of extracellular fluid according to the mannitol method of Schwartz, from such observations with inulin we also obtained greatly different values because of very slight differences in the position of 3 or 4 points not exactly on a straight line By using two of the points in preference to two others that were supposed to be on the same line a variation was obtained that was beyond our ability to correct or to interpret For accurate measurement of extracellular fluid the use of such a method which would certainly be applicable to and convenient in small infants has not been found useful

Professor JOSEPHSON (Stockholm) The decrease of extracellular fluid that you have found from the first eleven weeks on has interested me very much Cannot the difference between the behavior of inulin and that of mannitol which you have noted depend on changes in the permeability of the different cells to these molecules? Inulin and mannitol are of quite different size as are sodium and thiocyanate and the behavior of these two latter could be tested on erythrocytes for instance

Professor BARNETT (New York) I think you are quite right in implying that if we could demonstrate that not only these two electrolytes but also *substances as different as inulin and mannitol behaved in a parallel fashion* this would add strong evidence to the fact that the changes reflect differences in a space rather than in permeability of a membrane This was our initial thought when we attempted to determine inulin and mannitol spaces along with thiocyanate and sodium As I indicated with the method we were using we could not interpret the changes in inulin and mannitol spaces and we have not as yet been able to measure inulin spaces in small infants by the continuous infusion technique This becomes I think a technical matter of how good a pump one has Our mechanical pump will deliver fluids at the rate of 0 2 ml per minute However even 0 2 ml per minute for five hours in a small infant is a prolonged observation period which could easily change things Furthermore from our knowledge that extracellular fluid is greater in small infants the period of equilibration might be even longer in the small infant than in the adult

The changes in erythrocytes as a measure of changes in membrane permeability constitute a problem about which possibly Dr Hallman might speak Have we perhaps erroneously considered erythrocytes as such special cells that we have failed to explore sufficiently whether or not they provide any indication or evidence of what happens in other cells? This is a somewhat narrow point of view I know but there are many who think that the most special thing about erythrocytes is that they are readily available and can be easily studied

Dr HALLMAN (Helsinki) Erythrocytes are of course easily available but I think that they are not representative of all other cells Somebody— was it not Guest?—showed that in diabetic acidosis erythrocytes lose potassium

We in Finland have done some thiocyanate space measurements in severely dehydrated infants with gastroenteritis

In Figure 12 the round figures represent the individual cases On admission one would expect thiocyanate space to be below normal however in some case numbers such as 6 8 and 13 the thiocyanate space is higher than

normal amounting to almost 60 per cent of the body weight After treatment the thiocyanate space rose on every occasion We found no clinical evidence of edema Only after a few weeks did the range of thiocyanate space come back to normal It is hard to believe that the extracellular spaces were this big during dehydration and I think that there may have been a change in permeability of the cells

Professor BARNETT (New York) From your data and from those of Overman thiocyanate in diseased states certainly cannot be interpreted as

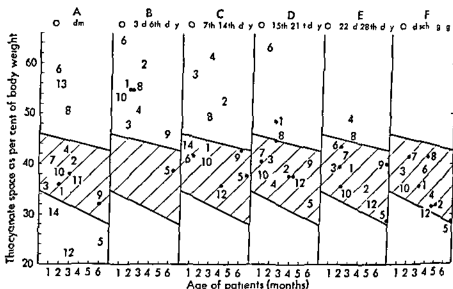


Fig 12 Thiocyanate (SCN) space at various stages of severe infantile gastroenteritis The abscissa shows the age of patients in months Normal values in lined areas (Hallman N and Kauhtio J *Acta paediat* 39 354 1950)

representing even changes in extracellular fluid much less an absolute measurement of it because the permeability undoubtedly changes not only in erythrocytes but as Overman has shown in other cells of the body too However I would hesitate to explain all of the unexpected findings in this way and I think it is important that we make such measurements in disease There is increasing evidence that dehydration as we see it clinically is not always due to a loss of extracellular fluid and we should not rule out the possibility that we may find instances in which dehydrated children have almost normal volumes of extracellular fluid

Dr SJÖGREN (Stockholm) How do the inulin and mannitol methods

compare in addition to the fact that inulin is easier to determine chemically and that mannitol distributes itself faster?

Professor BARNETT (New York) I think the advantage of the more rapid distribution of mannitol is a very great one and may become the most important one in terms of making measurements in young infants Mannitol however as I am sure you know has two disadvantages in regard to inulin other than the chemical determination which is more laborious though good First mannitol induces diuresis and therefore is capable of changing the physiologic state Second mannitol is metabolized for which a correction may have to be made These objections may be overcome by the shorter period of equilibration necessary in small infants

Dr EK (Stockholm) In considering the steady increase in extracellular fluid as measured by a single inulin injection do you assume a steady delay time or not? We have measured the delay time after single inulin injections and have found it to be steadily increasing which would appear to be due to continual mixing of inulin in the kidney pelvis If we fail to take this into account we are bound to get a steady increase in apparent extracellular space

Professor BARNETT (New York) When a measured amount of inulin is given the amount that has been excreted at the time that the concentration in plasma is measured must be known The delay time it seems to me would be a factor only at the time that one has assumed that equilibrium has occurred which is perhaps at the end of 2½ hours If delay time is not taken into consideration somewhat more than the amount measured has actually been excreted or somewhat less than the amount calculated is left in the body By the end of three hours the amount of inulin which is being excreted per minute is so much less than the amount that was being excreted during the first hours when the blood concentration was very high that even if we neglected delay time when the concentration is so low it would not influence the calculation of the space very much

Dr FRIIS HANSEN (Copenhagen) Could not this observation be explained as Dr Barnett put it in the beginning? After a single injection of inulin the blood concentration rises instantaneously and the concentration in the interstitial space is low The inulin diffuses rapidly into the interstitial space and is rapidly excreted but it will be excreted more rapidly than it diffuses After a given time the inulin is rapidly removed from the blood through the kidney and diffuses back from the interstitial space into the blood In that period the concentration in the interstitial space will be higher than in the blood because of the time lag and in that period we will have too low a blood concentration and thus too great a calculated volume

Professor BARNETT (New York) After equilibrium has been reached

there may be a point at which the level *in the blood* because of rapid renal removal is lower than the level in more remote extracellular spaces This would give a higher value for the inulin space than is true Although this is theoretically correct I do not know enough about the relative rates of excretion and diffusion to know to what extent it could account for these findings

Professor WALLGREN (*Stockholm*) *I will ask* Dr Friis Hansen to comment further on the subject we have just discussed and to give a little more of his own experience

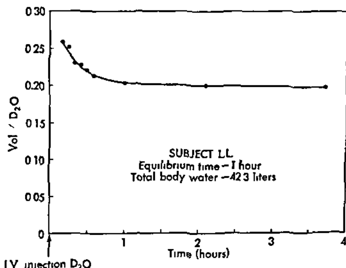


Fig 13 Serum deuterium concentration (venous blood) in relation to time after injection

Dr FRIIS HANSEN (Copenhagen) This work was initiated by Prof F D Moore surgeon in-chief at the Peter Bent Brigham Hospital in Boston During the time I spent there we first worked out methods and then made some determinations on normal adults later including children and old people

A known amount of heavy water is injected time elapses until equilibrium has been established the equilibrium concentration is determined and finally from the dilution the total body water is calculated

Figure 13 shows the concentration of heavy water found in venous blood at various times following injection After the intravenous injection the concentration decreases until equilibrium is reached in from one to two hours This equilibrium value is used for the calculation of total body water In animals we have found that equilibrium with cell water in the liver is achieved

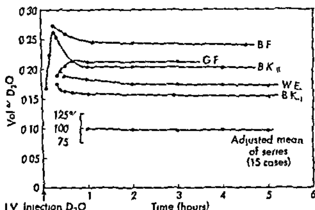


Fig 14 Serum deuterium concentration (venous blood) in four subjects in relation to time after injection

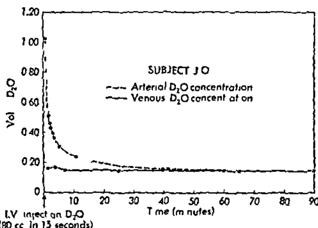


Fig 15 Serum deuterium concentration (arterial and venous blood) in relation to time after injection

in five minutes and that equilibrium with cerebrospinal fluid is reached in about ten minutes in the ventricles and in one hour in the lumbar region In humans we found that gastric juice is in equilibrium in 10 or 15 minutes But in abnormal accumulations of water such as ascites it takes about 6 hours until complete equilibrium is reached

Sometimes we do not get a steadily decreasing level after a single in travenous injection Figure 14 shows for instance the second curve steadily rising for the first half hour Sometimes we get a steep decrease and some times a curve that i [sic] almost flat

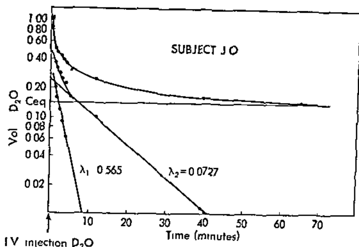


Fig 16 Serum deuterium concentration (arterial blood) on semilogarithmic plot analysis of slope components

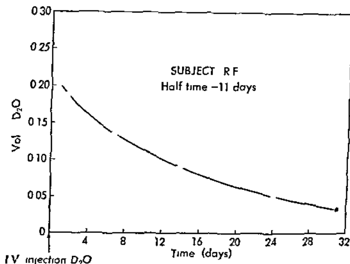


Fig 17 Serum deuterium concentration (venous blood) in relation to time after injection

In an attempt to explain this we made simultaneous determinations on arterial and venous blood and obtained the curves shown in Figure 15 Equilibrium is reached in about 60 minutes when the curves meet and stay horizontal This curve indicates that venous blood and arterial blood concentrations are quite different in the first hour and while curves for venous blood may have various shapes those for arterial blood show exactly the same

configurations in all our experiments Some of the difficulties which arise in the one injection technique in the determination of extracellular space with inulin or mannitol may be accounted for by this difference between arterial and venous blood concentrations The venous blood reflects only a small fraction of the total body and is dependent upon the local rate of transfer in that particular extremity where it is drawn It has also been proved that in determination of the PAH clearance the arterial and venous concentrations are so different that it may be necessary to make a correction

Figure 16 shows how the arterial curve can be represented on semi logarithmic paper where it is broken down to two straight lines of two different slopes indicating that there are two major rates of transfer These two slopes are practically the same in humans and in dogs

Figure 17 shows a disappearance curve over a period of days The half time of heavy water in the body is about 9 days in an adult in a small child it is from 3 to 4 days

Total body water determination in children was carried out by simultaneous use of the antipyrine and heavy water methods Figures 18 and 19 show that the highest value for body water is found in the first 10 to 14 days of life when water constitutes about 80 per cent of body weight Then there is a gradual decrease during the first 6 months from about 80 per cent to between 55 and 65 per cent In adults we found a variation between 58 and 68 per cent with an average of 62 per cent in the male group and in females the fluctuation was from 48 to 58 per cent with an average of 52 per cent This is presumably because of a higher fat content in females since fat has very low water content

Figures 20 and 21 show the relationship between body weight and water content For infants up to 10 kg there is a very close correlation between the two but in bigger children the correlation is not so good As a result one of the great problems is that if we make a single determination on a sick infant we are not able to tell whether the total body water is low or normal or to what extent it is lowered This is due to the fact that it has been very difficult or even impossible to find a close correlation between any physical indices and the total body water so that the variation between two normal children may be even larger than the pathological changes in one child We have tried to correlate body water to the surface area to the three-quarter power of the weight and to other physical indices that have been used to compare the metabolic rate of different subjects and animals The closest correlation is found between body water and surface area as seen in Figure 22

Thus heavy water is a good tracer which mixes completely with water

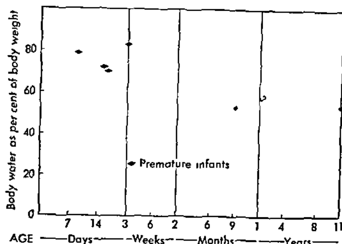


Fig 18 Body content of water in infants in relation to age (Friis Hansen B J Holiday M Stapleton, B M and Wallace W M "Total Body Water in Children" *Pediatrics* 7 324 1951 Charles C Thomas Publisher)

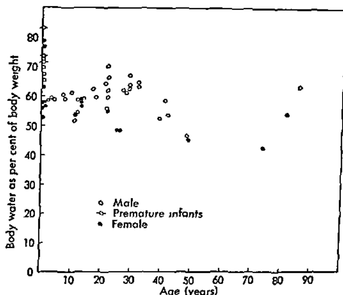


Fig 19 Body content of water in relation to age

The organism does not distinguish between ordinary and heavy water Heavy water at low concentration is found as DHO It is evenly distributed throughout the body and there is rapid equilibrium There is no need to correct for losses because after ten minutes the concentration is practically constant and any loss taking place thereafter will not change the result

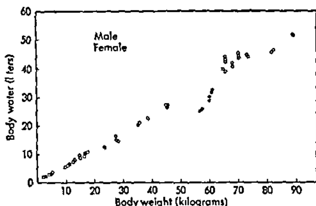


Fig 20 Relation of body water to body weight

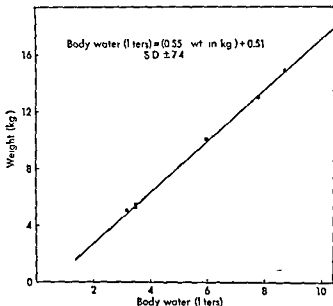


Fig 21 Relation of body water to body weight in infants and children (Friis Hansen B J Holiday M Stapleton B M and Wallace W M "Total Body Water in Children" *Pediatrics* 7 325 1951 Charles C Thomas, Publisher)

Determination of heavy water which makes this method feasible is carried out either by a densimetric method or in the mass spectrometer For an ordinary clinical laboratory densimetry by the falling drop method or the gradient tube will be the easiest An accuracy of 1/1000 per cent of a volume per cent of heavy water is attainable That means that the total body water can be determined to about 1 per cent

For the falling drop method the theory is that we measure very accurately the time taken for a drop to fall through a nonmiscible liquid such as ortho fluorotoluene

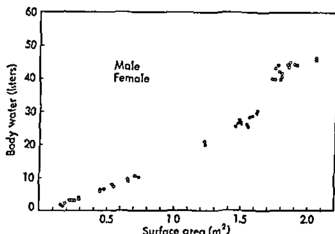


Fig 22 Relation of body water to surface area

Dr Ussing has worked with the gradient tube and might comment on this

Dr USSING (Copenhagen) We have found that it is possible to use the gradient tube and to obtain practically the same accuracy without the very exacting timing work which makes the use of the falling-drop method so tiring Moreover the gradient tube method has the advantage of temperature constancy not being so critical We use relatively large drops usually 10 micro liters With that size the risk of the drop being contaminated and of its density thus being changed is lessened The other advantage of using the larger drop is that we can then use a gradient tube with a less steep gradient For instance it is possible to use a gradient where the upper liquid has a density say of 0 997 and the lower liquid a density of 1 004 The difference is thus only seven in the third decimal place One thousandth of a per cent makes a difference in the level where a drop stops of about one twentieth of a millimeter a difference which can be easily read with the microscope The drop has attained equilibrium in 10 minutes The reading can be repeated as often as needed since the drop remains in place for hours

Professor BARNETT (New York) I should like to emphasize the question referred to by Dr Friis Hansen of what standards of reference should be used in disease or in changes in normal children with growth to correct any of these physiologic data I think this problem needs to be much more thoroughly explored We shall come back to it in talking about kidney function in young infants because it is very important there

FLUID AND ELECTROLYTE LOSSES IN DEHYDRATION AND THEIR REPAIR

Professor BARNETT (New York) Advances in our knowledge of how to treat infants who have abnormal losses or retention of water and electrolytes have been based on several related and well-defined areas of increased physiologic understanding These include (1) knowledge and application of the knowledge of the transfer of water and electrolytes particularly sodium and potassium between intra and extracellular fluids (2) a more complete understanding of the volumes of body water compartments (3) the recognition and consideration of the effect of reduced kidney function in young infants and (4) the increased knowledge of hormonal regulation of water and electrolyte distribution and excretion

Despite the very great improvement that has been made in our treatment of dehydrated infants I think we must now depart from the idea of standard solutions Hartmann s Darrow s and Butler s solutions to mention three that are in common use in the United States have very great value but a better approach to the proper treatment of an individual infant who has diarrhea and has lost water and electrolytes must be based on a more quantitative estimate of how much water sodium potassium chloride bicarbonate calcium phosphorus and perhaps other things this particular baby has lost This kind of individualization in our thinking and in our treatment is the next logical step It would almost be necessary in order to apply all of our knowledge to the treatment of a given infant to have had the infant on balance studies for a week before it got sick and then during the few days it was sick At the end of this time we could say that *this* is how much *this* baby needs for repair Granted that this is not possible it is however possible for us to think of the infant in terms of what we might have found if the baby had been on balance study For example the amount of water that a dehydrated baby requires should be considered not as 150 or 200 ml per kilogram but more properly in terms of the amount of weight lost as judged from the weight before the infant got sick and the weight at the time of treatment

Of recent developments the one that I think may be of the greatest interest and perhaps greatest importance to consider is the role of potassium losses in dehydration and the proper correction of these losses

A great deal of physiologic information concerning potassium metabolism is rapidly accumulating This is partially accounted for by the fact that a good practical and rapid method for determining potassium has been devel oped in the past few years With this new method there has been increased interest in the studies of many years ago that suggested but failed to prove quantitatively that potassium was handled by the kidney by a process of filtration absorption and tubular secretion

Circumstances which may result in potassium deficiency and which should be considered in evaluating the potassium status of a sick infant are the fol lowing as pointed out by Elkinton and Tarail urinary excretion of potas sium continues to occur in the absence of intake of potassium and the ratio of urinary potassium to plasma potassium rarely falls below one There is evidence that during water diuresis this may occur but in general it is true that urinary excretion of potassium continues even in the presence of marked depletion of total body potassium Furthermore urinary excretion is rapidly accelerated by dehydration itself and also by tissue trauma

The second circumstance which in combination with the first may lead to marked loss of potassium is abnormal loss of gastrointestinal secretions by vomiting or gastrointestinal suction or from intestinal fistulae or diarrhea

Third abnormal losses can result from excess of adrenocortical steroids as when DCA is administered or supplied endogenously as in Cushing s disease

Finally potassium is lost in excess through the reciprocal relationship which obtains under certain well-defined conditions between intracellular potassium and extracellular bicarbonate

Conditions which result in deficiency of total body potassium are not the only ones which lead to low serum potassium and we must consider circum stances which may result in hypopotassemia separately For example with out any *change in total body potassium rapid expansion of extracellular fluid* which may increase by as much as 100 per cent would in the absence of adjustments reduce serum potassium by 50 per cent The level of potassium in serum may also be influenced by transfer of potassium between intracellular *and extracellular fluids* without changes in total body potassium The most striking example of this which has long been known is the low serum potas sium observed in familial periodic paralysis which is associated with decreased potassium excretion and with transfer of potassium from the extracellular to intracellular fluids Similarly the administration of glucose and insulin can lower serum potassium without decreasing total body potassium since potas

sium is stored with glycogen in a ratio of about 36 millimol of potassium per gram of glycogen laid down in the liver Finally testosterone through its protein anabolic effects can also cause transfer of potassium from extracellular to intracellular fluids

I think it is worth emphasizing a converse to the above in the presence of low total body content of potassium the concentration of potassium in serum does not afford a good estimate of what the total stores of potassium are A brief example of what happens in diabetes may illustrate this point

The vomiting polyuria and dehydration of a child going into diabetic acidosis will all lead to excessive loss of urinary potassium to which will be added the reduction of intake because of vomiting There are therefore many reasons to account for the depletion of total body potassium However at the time we see children with dehydration and acidosis from diabetes the very rapid transfer of potassium from intracellular to extracellular fluids which accompanies the interruption of carbohydrate metabolism may have produced a situation in which the rate of entrance of potassium into extracellular fluids has exceeded the accelerated loss It is not uncommon to find serum potassium high or more frequently normal in untreated patients with diabetic acidosis at a time when the whole body shows marked potassium depletion

During treatment of such a patient without potassium the following events occur which can lead to hypopotassemia (1) there is rapid expansion of the extracellular fluid with the administration of fluids (2) with correction of dehydration the urinary excretion increases very rapidly and (3) potassium is deposited with glycogen after treatment with insulin It is during treatment that serum potassium may fall to low and sometimes dangerous levels

The same events occur in diarrhea which we can discuss more fully when we consider the solutions used

The circumstance in which we have seen hypopotassemia most frequently and in which we have found parenteral administration of potassium most useful has been in infants who have had gastrointestinal operations and who have been off feeding by mouth for periods of two three or four days In addition these infants have frequently had gastric suction and the special conditions which were defined as necessary for the development of the relationship between extracellular bicarbonate and intracellular potassium have obtained The patients are usually given adequate water sodium and chloride but no potassium In these circumstances which are exactly those which Darrow produced experimentally we have seen many instances of marked elevation of serum bicarbonate The administration of potassium to such infants whose extracellular bicarbonate has been as high as 40 millimols per liter has

resulted without any acid administration in a fall from 40 to normal values of 25 millimols per liter in a period of 24 hours We have therefore in our clinical experience confirmed as other people have the importance of Darrow s experimental findings on the reciprocal relationship between intracellular potassium and extracellular bicarbon ite

The clinical diagnosis of potassium deficiency and the evaluation of treatment with potassium are difficult Poor muscle tone and weakness are observed so frequently in babies who have diseases which produce these deficiencies that it is difficult to evaluate the effect of inclusion of potassium in treatment The diagnosis of potassium deficiency is made (1) from the history by knowing the conditions which produce it and less from examination of the serum potassium which may be and often is normal at the beginning of treatment (2) from the electrocardiogram (3) from the elevation of serum bicarbonate and (4) from the important fact that when we administer potassium to a person who is not deficient in potassium the potassium is not retained Retention of added potassium by the body is perhaps the best evidence we have that there was depletion in the potassium but this fact is not of direct practical aid

The important problem of hyperpotassemia is limited almost exclusively to instances of excessive administration to patients with reduced kidney function In young infants kidney function may be sufficiently reduced as a result of incomplete development or dehydration so that generally we have not treated such infants with parenteral solutions of potassium during the first day or so By the time we have wanted to start potassium therapy the infants have usually been at least on fluids by mouth and we have given them potassium orally in the form of potassium chloride or preferably orange juice and milk

The symptoms of hyperpotassemia are primarily cardiac and seem to be due to the high serum potassium rather than to high intracellular potassium in cardiac muscle The diagnosis again is from recognition of the disturbances which lead to it Here the serum potassium is more helpful because it is unlikely that excess total body potassium will be found in the absence of high serum potassium The electrocardiogram is again helpful

The treatment of hyperpotassemia is not firmly established but deserves mention In experiments calcium has been shown to protect animals against toxic doses of potassium administered intravenously The exact mechanism is not known and the effect has not been demonstrated in patients

The administration of glucose and insulin accelerates the transfer of potassium from extracellular to intracellular fluids and has been shown to be of benefit in cases of high serum potassium

Other measures which have been advocated include the use of the artificial kidney peritoneal lavage and more recently the administration of cation exchange resins

Dr FRIIS HANSEN (Copenhagen) In order to study the rate of transfer of potassium from the cells to the extracellular space in Dr Moore s laboratory we tried to study the equilibration time of radioactive potassium given intravenously We found that it took about 10 hours to reach complete equilibrium with the cells Of course this is done under normal conditions and does not prove how fast the potassium moves in and out under pathologic conditions But if we take this 10 hour value it also gives some indication of the rate at which potassium can be administered because we must expect it to take about 10 hours before all of it has penetrated into the cells

Professor BARNETT (New York) I would like to be sure that I am right in understanding that if a tracer dose of potassium is administered it takes as long as 10 hours to reach equilibrium as judged from when the volume of distribution becomes constant

Dr FRIIS HANSEN (Copenhagen) Yes to the time when the specific activity is constant

Professor BARNETT (New York) This is considerably longer than the time required for sodium

Dr FRIIS HANSEN (Copenhagen) Yes because equilibrium between sodium and radioactive sodium within the extracellular fluid in this experiment would be reached almost as fast as with water that is within one hour or so But as Dr Barnett also pointed out attainment of equilibrium with the total sodium which includes the sodium in the bones and cells requires 10 to 20 hours

Professor BARNETT (New York) How accurately does the equilibrium distribution of radioactive potassium or sodium represent actual transfer of carrier potassium? It seems to me that equilibrium depends on two things (1) exchange of radioactive potassium and (2) transfer of total potassium from intracellular to extracellular compartments The former may not give a really true measure of the rate of transfer of potassium into cells Is this correct?

Dr USSING (Copenhagen) The question depends on whether you inject measurable amounts of potassium or not If you inject carrier free potassium the whole problem is very simple You have a certain mixing rate and from that you get a measure of total body potassium But if in injecting the radioactive material you inject a chemically significant amount of potassium then there will be a redistribution of potassium in the cells and as Dr Friis Hansen mentioned that process will take a long time If you want to

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measure potassium by dilution you should use as far as possible carrier free potassium

Professor BARNETT (New York) This observation is certainly confirmed by the results of balance studies which indicate how much potassium can be retained over a period of time Even in the presence of marked depletion of intracellular potassium it has been shown that potassium can be replaced without raising extracellular potassium at a very slow rate actually taking several days or a week or more These observations can be explained by the slow rate of movement into cells which Dr Friis Hansen mentioned It would be interesting to know if the rate of achieving equilibrium with radioactive potassium is influenced by differences in concentrations of intracellular potassium

Professor WALLGREN (Stockholm) We will now close the morning session and discussion will be resumed in the afternoon

Professor BARNETT (New York) It might be well to start this afternoon with some observations on the effect of adrenocortical hormones on electrolyte distribution and excretion

This is a very abnormal situation but it can serve as an introduction to the discussion of normal hormonal control of water and electrolyte distribution I am particularly interested in presenting these data because I think they may indicate that the response of young infants to adrenocortical hormones may be different from that of adults although this interpretation is not by any means proved

These data were obtained in a child with the curious combination of adrenocortical insufficiency and virilism This disease was first recognized and described in 1939 by Butler and Talbot in Boston and Wilkins and others at Johns Hopkins It is apparently due to bilateral adrenal hyperplasia occurring during fetal life in males It seems to be comparable to the type of change in the adrenal which in the female produces pseudohermaphroditism In the male however it produces no signs of any effect of excess androgenic activity at birth but is in a large percentage of instances associated with signs of adrenal insufficiency particularly of the electrolyte-controlling hormone of the adrenal The signs of virilism do not usually become prominent until 6 months or so of age Consequently before the nature of the disease was recognized most of the children had died of unrecognized adrenal insufficiency before virilism had appeared It seems therefore that whereas we previously thought that congenital bilateral adrenal hyperplasia was a

disease limited almost exclusively to females and producing pseudohermaphroditism the actual fact is that it occurs perhaps as frequently in males

One other important clinical fact about this disease is that it occurs in several children in the same family in a very high percentage of instances In at least half of the reported cases in which there were siblings other male siblings had the same disease or female siblings had pseudohermaphroditism

My purpose in presenting the problem of adrenocortical insufficiency with virilism in this discussion of water and electrolyte metabolism is to consider the effect of adrenal insufficiency and of desoxycorticosterone acetate administration on potassium metabolism The commonly accepted belief of the way in which potassium is affected by adrenal insufficiency is that as an experimental animal or patient with Addison s disease becomes insufficient the primary event is a decreased renal excretion of potassium associated with increased renal excretion of sodium and that as a result of decreased excretion there is retention of potassium with elevation of serum potassium and increase in total body potassium Treatment with DCA has been thought to reverse these changes producing primarily increased potassium excretion associated with a lowering of extracellular potassium and finally a decrease in total body potassium which with prolonged treatment can be shown in experimental animals to be associated with toxic signs and symptoms similar to those that are produced by feeding diets low in potassium The actual problem presented was twofold how to treat such a child who could be expected to need desoxycorticosterone throughout life whether this need would produce dangerous potassium depletion

I have photographs of one of three such infants whom we have observed In this child (Fig 23) whose history was characteristic of the others symptoms had begun very soon after birth and had been misdiagnosed as pyloric stenosis which frequently happens The child was extremely dehydrated and malnourished and was finally diagnosed by the fact that ordinary amounts of water sodium chloride and glucose failed to relieve the dehydration until first adrenocortical extract and then desoxycorticosterone were given The diagnosis was confirmed by recurrence of his symptoms when the hormone was withdrawn

The next picture was taken just a few weeks afterward (Fig 24) when he had been treated with desoxycorticosterone and we can see in this picture that there is a suggestion of enlargement of the penis and wrinkling of the scrotum which by the time he was six months old was very obvious clinically His 17 ketosteroid excretion was 5 mg per 24 hours which is about the expected value for preadolescent males

A series of metabolic observations is shown in Figures 25 26 and 27 *During an interval when he was well controlled and thriving on 2 mg per day* of desoxycorticosterone acetate with 3 5 gm of sodium chloride added to his formula and 0 8 gm of sodium lactate he was *in positive sodium balance* These balances are plotted with the height of the column indicating the intake



Fig 23 Adrenocort cal insuffi c ency and v rilism before treatment



Fig 24 Adrenocort cal insuffic ency and viril sm after treatment

and urinary plus stool excretion being plotted down from the top A clear space above the line indicates a positive balance and an extension of the col umn below the line a negative balance The changes in sodium balance and excretion and serum sodium were those that were anticipated When first DCA was withdrawn during the second period and later when the salt intake was reduced sodium excretion increased and the balance of sodium became negative This was accompanied by a fall in the concentration of sodium in

the serum These changes were reversed as anticipated when DCA was replaced in the fourth period during which he received 5 mg on the first day and 2 mg thereafter (Fig 25)

These changes then were those that would be anticipated from other observations on the effect of desoxycorticosterone acetate The changes in potassium however were quite unexpected

Here if the concentration of potassium in the serum is followed we see

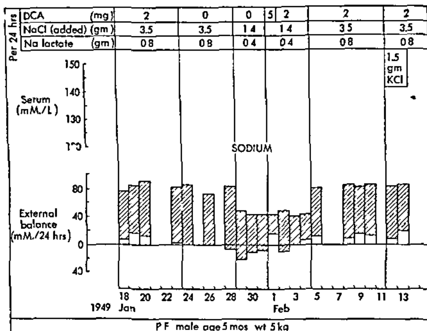


Fig 25 The effect of DCA and salt therapy on external sodium balance in an infant with adrenocortical insufficiency and virilism

that with withdrawal first of DCA and later of sodium there was a prompt elevation of serum potassium from values around 5 to a value as high as 8 mM /L This value returned promptly to normal with the reintroduction of treatment with DCA and sodium chloride (Fig 26) This also was as anticipated However when we look at the balances of potassium we see that during treatment with DCA he was in positive potassium balance When DCA was withdrawn and later when salt was decreased instead of potassium retention occurring potassium excretion increased as serum potassium increased When DCA was replaced accompanying the fall of serum

potassium there was a decreased excretion of potassium and he went back into positive potassium balance

During the last period the potassium intake was increased There was no increased retention of potassium which as we discovered this morning is a good indication that there is not depletion of intracellular potassium because if there had been we would have expected him to retain potassium

When intracellular balances were calculated according to the method of Darrow it was seen that during the period of insufficiency there was a marked

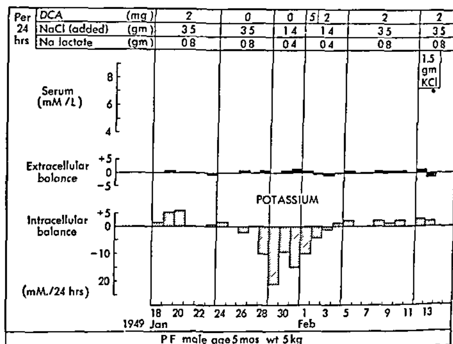


Fig 26 The effect of DCA and salt therapy on potassium balance in an infant with adrenocortical insufficiency and virilism

shift of potassium from intracellular to extracellular fluids and here again it is shown that there was no increased retention of intracellular potassium during the addition of 1 5 gm of potassium chloride to his diet

The changes in kidney function during these periods are shown in Figure 27 which I shall only discuss briefly because we shall have another opportunity to discuss these in more detail in a later panel No fall in glomerular filtration rate as estimated from 24 hour clearances of creatinine was observed perhaps because only a mild degree of insufficiency was allowed to

develop The unexpected effects of adrenal insufficiency and of DCA administration may perhaps best be explained by the possibility that excess production of androgenic hormones influenced the action of electrolyte controlling hormones There is experimental evidence that this does occur

However if the evidence is critically reviewed the generally accepted concept of the effect of adrenal insufficiency and DCA on potassium excretion

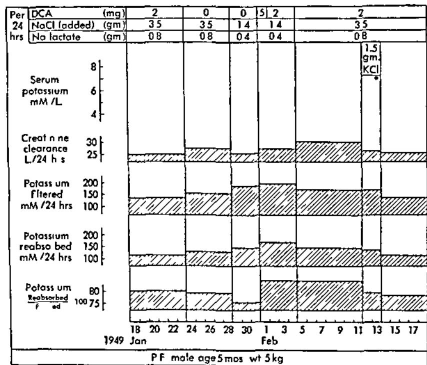


Fig 27 Effect of DCA on renal function in infant with adrenocortical insufficiency and virilism

in normal individuals is not as well established as is thought The third possibility for the unexpected findings is that young infants respond in a different way than adults do to adrenal insufficiency and to DCA

Dr FRIIS HANSEN (Copenhagen) Do you think that potassium regulation due to the cortical steroids is primarily a kidney effect and secondarily a shift from the cellular to the extracellular phase?

Professor BARNETT (New York) It has been generally accepted and is written in textbooks that the primary effect is on renal excretion of potas

sium Viewing the evidence critically as to whether DCA acts primarily on potassium excretion I do not find it very convincing in any of the published reports I think the recent study of Levitt and Gaudino on changes in body water compartments and on distribution of radioactive sodium and potassium makes it even more unlikely that the sole effect of the electrolyte hormones in the adrenal is on renal excretion They point much more toward an effect on actual water movement and perhaps transfer of electrolytes across membranes either influenced by changes in the membranes themselves or secondary to the movement of water Dr Ussing has made many observations on factors that can effect the transfer of ions across membranes

Dr USSING (Copenhagen) There are many observations to the effect that potassium is mostly a passive ion in the nerve and in the muscle and probably in frog skin so that the potassium movements are only secondary effects of the sodium transfer

PITRESSIN RESISTANT DIABETES INSIPIDUS

Professor BARNETT (New York) A rather interesting small series of patients has been described recently in whom from birth on there appears to have been a moderately severe type of diabetes insipidus These cases have occurred sporadically in both males and females They have been described as occurring in families where the disease has manifested itself only in the male members but has been transmitted by the females through as many as three generations in the case reported by Williams From the observations that have been made in the carefully studied cases this disease appears to be a true end organ defect in the response to antidiuretic hormone It appears to be one of the very interesting types of selective defective response of the tubules because other functions of the tubules insofar as they can be measured seem to be within normal limits These children begin to show symptoms of polyuria very soon after birth and before they are recognized the children have often had long periods of unexplained dehydration sometimes associated with fever The changes in electrolyte excretion have been irregular In the cases reported by Waring there was always marked elevation of serum sodium and chloride accompanying the dehydration and fever although this was not true in the case that was carefully studied by Dancis at the Bellevue Hospital in New York The evidence that it is a true tubular defect stems from several observations In the first place the polyuria persists in the absence of increased fluid intake If fluid is restricted the polyuria continues and dehydration occurs This tends to differentiate it on clinical grounds from so-called psychogenic drinking in childhood and also from the

so called adrenal or DCA type of diabetes insipidus in which with restriction of water there is usually complete relief from the polyuria

In all of the cases the polyuria has shown no response to very large doses of Pitressin It was demonstrated in one study that the serum of the patient did not inactivate Pitressin and it was also shown by Dancis that there was a large amount of antidiuretic hormone in the urine of this patient Insofar as one can accept the view that antidiuretic hormone present in the urine is identical and derived from the posterior pituitary gland it would seem to complete the evidence that we are dealing with a failure of the tubules to respond to a normally functioning posterior pituitary antidiuretic mechanism Clearances of mannitol and PAH and normal maximal tubular excretory capacity for PAH in the infant reported by Dancis support the evidence that this is a specific malfunction of the tubules Insofar as one could evaluate the capacity of the tubules for reabsorption of sodium chloride and phosphate this seemed to be normal in the infant

I think there is not much more one can say about these infants except that there does exist this type of defect which can be related to a failure of development of the renal tubule The tubule of the newborn infant is relatively unresponsive and these children may represent a developmental failure rather than any actual disease of the tubule

Dr USSING (Copenhagen) I would like to discuss briefly the functions of the isolated skin of amphibians even though it may seem a problem far removed from today s subject It has been shown that the skin of the toad and the frog responds to posterior lobe hormone in exactly the same way as does the kidney we get an increased absorption of water through the skin by adding minute amounts of posterior lobe hormone Therefore I think that even though the organs are different the mechanism by which the hormone acts upon them may be the same

My story is rather involved but may I give a few indications of what we have found? When we short circuit the frog skin the total current we can draw from it comes from the active transport inward of sodium Further it can be shown that the proportion between influx and outflow of sodium as determined with radioactive tracers is a measure of the potential acting upon the ion The difference between influx and outflow on the other hand is the sodium-current strength From these two figures—that of the potential expressed in volts and that of the net current expressed in amperes—we can determine the resistance to the particular ion under study

When adrenaline is added to the skin we get a drop in the potential carrying sodium through and also a drop in the resistance to sodium Posterior lobe hormone on the other hand has no effect upon the transporting poten

tial It acts solely by decreasing the resistance to sodium The effects of adrenaline and of posterior lobe are additive Since adrenaline is most likely to act upon the cell membrane proper it practically follows that the posterior lobe hormone must act upon some layer other than the cell membrane

It is tempting to assume that in those patients where no effect of the posterior lobe hormone is observed there is a maldevelopment of that particular layer which is sensitive to the posterior lobe hormone

Professor BARNETT (New York) Can these observations in an artificial system have any bearing on the response of the kidney to an osmotic load during hydropenia when maximum antidiuretic hormone release and maximum reabsorption of water can be assumed? If at this time a large osmotic load is given the urine volume increases and this is associated with a fall in the total osmolarity of the urine This increase in urine flow is uninfluenced by antidiuretic hormone since it occurs in the presence of maximum antidiuretic hormone response This is a physiological system and I wonder if it could be explained by any of your observations of frog skins

Dr USSING (Copenhagen) I would prefer to discuss this problem later I do not feel justified in drawing any conclusions at present

Professor WALLGREN (Stockholm) Is there any way Dr Barnett by which you can distinguish this type of patient from other patients with polyuria except for persisting polyuria when fluids are restricted?

Professor BARNETT (New York) There is a test which was devised by Hickey and Hare which I think is probably familiar to all of you but it might be worth briefly describing because in addition to providing a good diagnostic differentiation for causes of polyuria it is also an elegant demonstration of the mechanism of antidiuresis in the presence of dehydration

This test consists of inducing a water diuresis by administering 20 ml of water per kilogram of body weight over a period of an hour to a subject who has polyuria of unknown cause The urine volume is measured at 15 minute intervals during this hour and at the end of the hour an intravenous infusion of hypertonic sodium chloride (2 5 per cent) is given at the rate of 0 25 ml per kilogram per minute for 45 minutes In the presence of overhydration the increase of total osmotic pressure of the plasma through the mechanism of the osmoreceptors described by Verney and Hare and by others produces a very prompt fall in rate of urine flow

Figure 28 shows the results of the test in two adults and in a child in our clinic It can be seen that after the first 15 minutes of infusion there is a rapid decrease in the rate of urine flow from values as high as 15 to 20 ml per minute down to 1 and 2 In the bottom graph we had given 40 instead of 20 ml per kilogram for hydration and the response was somewhat delayed

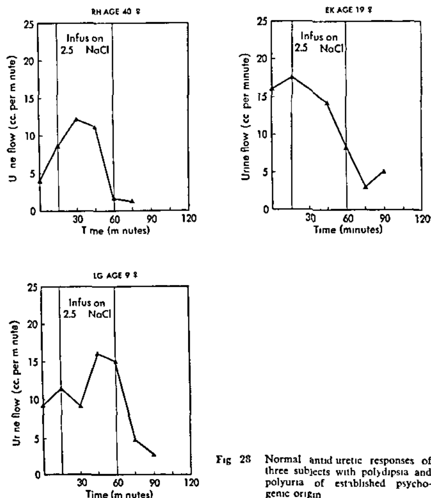


Fig 28 Normal antid uretic responses of three subjects with polydipsia and polyuria of established psychogenic origin

The three curves shown in Figure 29 are characteristic of various re sponses The solid curve at the top indicates the response in a patient with true diabetes insipidus in whom the water diuresis was not affected by the infusion of hypertonic salt but who showed a very prompt response to injected Pitressin at the end of this period the other curves represent normal responses

A patient with kidney disease or a child such as those we have been dis

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cortical hormones and the posterior pituitary hormone in regulating urine flow appears to be a very complicated and uniquely balanced system The article of Gaunt on the adrenal control of water excretion which has recently appeared is an excellent survey of our present incomplete knowledge of the effects of these two systems on water excretion

REFERENCE

Gaunt R Birnie J H and Eversole W J Adrenal Cortex and Water Metabolism *Physiol Rev* 29 281 1949

cussing with Pitressin resistant diabetes insipidus would fail to show the fall in urine flow either with the infusion or during the injection of Pitressin

Professor LEVINE (New York) Thank you It is interesting that this test was I think originally described by Dr Hare and called the Hare test in the United States Dr Carter who applied the test after it had

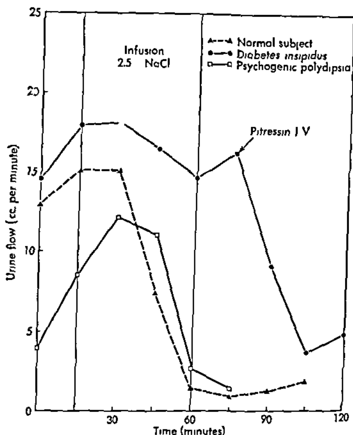


Fig 29 Antidiuretic responses in three subjects

been described had her name attached to it in Holland where it is called the Carter test

Dr FRIIS HANSEN (Copenhagen) May I ask Dr Barnett whether he has had any experience with the use of any of the water tests?

Professor BARNETT (New York) No we have not used these to any extent The experience in very young infants who show not only an inability to concentrate but also slow excretion of a water load would seem to be directly related to this The very fascinating relationship between adreno

through the gum because we knew that rickets is associated with delayed dentition We have made roentgenological examinations of the wrist and ankles at four week intervals in order to have a record of the rate of development of carpal centers and to ensure that no rickets was present We also noted rates of developmental achievement which in infancy are muscular achievements because it is known that rickets is associated with lack of full development of the musculature and also because we wished to ensure that we were duplicating home environment as nearly as possible in giving each infant the same stimulation he would receive were he at home Muscular achievements are a record both of the nutrition and of the emotional stimulation of the child The most important check was the clinical appraisal of the child at the beginning and at the end of the study

This method of study is necessarily very slow We can study a maximum of only twelve babies a year and it is a long and laborious task to collect sufficient data There were many questions to be answered among the first of which was how many collection period studies we could obtain from each infant In any method where excreta of infants are collected the infant must necessarily be restrained to some extent It was necessary to study the effect of such restraint of the infant upon the development of fatigue as an excessively tired baby is not a normal baby

A normal full term infant particularly an older infant moves about a great deal and to restrain such a baby for a long period results in unnecessary fatigue and unhappiness We measured the amount of fatigue by studying the length of daytime sleep after the periods of restricted activity In order to eliminate the factor of fatigue it was found necessary to limit the period of study to three consecutive 24 hour periods Therefore the younger infants were studied for 72 hour periods on alternate weeks the older infants for similar periods two weeks out of three The infants have shown no deleterious effects under this regimen that is no infant has shown signs of undue fatigue and no infant has forgotten a newly learned muscular achievement because of the period of restraint The recent studies showing the deleterious effects of muscular inactivity (bed rest) on the retention of calcium and phosphorus by adults have corroborated our concern in avoiding unusual restraint for the infants we have studied

The shortness of the collection periods has resulted inevitably in a wider range of data than might have been obtained had we used say 10-day collection periods But we feel that the collection errors are compensated by the larger number of studies possible for each infant and that the results are far more characteristic of the healthy baby than they would have been had we used longer periods of collection

CHAPTER II

Panel on Calcium, Phosphorus, and Vitamin D

STUDIFS ON THE NUTRITIONAL REQUIREMENTS OF INFANTS

Professor STEARNS (Iowa City) This afternoon I shall tell you a little of our philosophy and plan of study the details of our results I shall leave for later

All the work on which I shall report here has been a collaboration between Dr Philip Charles Jeans our professor and head of pediatrics and myself We started our study with this postulate in order to know the requirement of any substance in infancy it would be necessary to study the child at least throughout the major part of the period of infancy because we know that the effects of malnutrition may be delayed before they can be recognized clinically A second precept was of course that laid down by all students of nutrition that no infant can be subjected to a dietary regimen known to be undesirable that we must only work toward the betterment of and not in any way study the effect of malnutrition on infants We have studied only infants who were clinically normal at birth Our definition of requirement was what was needed for good growth and development And because the goal—the perfect infant—was unknown and is still unknown and will probably always be unknown it seemed to us that we should have as many methods of evaluating growth and development as possible

So in addition to retention studies and the usual hospital records of weight temperature excretion and so on we have made records of the changes in serum calcium phosphorus phosphatase serum protein and hemoglobin values We have determined the rate of growth in height at biweekly intervals We have recorded the date when the tooth could first be felt erupting

the buttocks rest Otherwise the baby can kick as usual The infants are very securely tied at the hips We have a wide canvas band lined with soft material which is pinned around the baby s thighs and then securely pinned to the canvas mattress A part of this T band is carried back over the top of the bed and fastened to a crossbar these bands keep the baby from sliding down into the hole through which fecal collections are made This setup is



Fig 30 The undraped metabolism bed showing washable canvas top stockinet restraining bands for the feet canvas restraining band for the thighs and the small mattress for the baby s head The bottle used for collection of ur ne and the dish for the collection of feces are vis ble Note that the bed is so hinged that either end may be raised



Fig 31 The undraped metabolism bed with boy infant 38 weeks old showing urine-collecting ap paratus in position A 6 in glass or porcelain dish is placed on the inverted glass jar close under the buttocks for collection of feces The illustration shows the method of fastening the restraining bands and the degree of free dom permitted the infant

for a boy infant we use evaporating dishes or Pyrex dishes for collection of the feces and a bottle for collection of urine When the beds are used for the older children they are usually used only at night but may be used con tinuously if the child is not dependably trained

Figure 31 shows a male infant on the frame you can see the stockinet band at the ankle and the wider band at the thigh Because with older and very active children a glass adapter for the collection of urine results in stasis and ed ma we have evolved a very simple collection apparatus made up of ad hesive tape and a rubber glove finger which works very well and is extremely

Infants are selected for the study as soon after birth as possible We often take them directly from the maternity ward to our service Our source of infants at first was wholly from the child placing institutions that is infants who were born to unmarried mothers and who would later be placed for adoption At present we are studying infants from a wide variety of nutritional backgrounds Some of them are still from the first category and others are infants born of mothers with chronic illness particularly tuberculosis where it is difficult for the family to provide care for the newborn infant At present the largest group of our infants come from our student body They are babies born to married students whose mothers often are in school and our care for the infant permits the mothers to finish their education In other cases it has been necessary for the mother to earn a livelihood in order that the father can continue his education We feel very proud that our reputation in our own community is such that even the medical students are willing to entrust their infants to our care

When the dietary regimen is decided on the amount of vitamin to be studied is kept to a constant daily dosage throughout the entire study Otherwise the diet is graded according to the age of the child We give the milk formula vitamin D and orange juice or some other form of vitamin C from birth Then at three months of age we add iron to the feeding formula At four months we add sieved fruit and at five months a sieved vegetable At about eight months of age the children are given a mixed diet But the amount of vitamin D they are given and its method of administration remain constant throughout the entire period they are under our care The amount of the formula taken is decided by the baby We have found no good means of changing his mind The formula and the supplementary foods are always made up in such an amount that an aliquot is sent to the laboratory for analysis Thus any error in the diet kitchen is carried through to the laboratory and the formula that we analyze is a part of the actual formula that the baby gets Our methods of analysis are all standard macromethods for the urine feces and diet

I would like to show you the metabolism bed used for the collection of excreta from normal full term active infants which must be quite different from that used for small inactive infants

Figure 30 shows the frame of the metabolism bed as a skeleton It is merely a wooden frame so hinged that either end can be raised either to prop up the baby or to get at the equipment A canvas top which can be removed and washed conveniently is laced down to the frame A small flat pad is placed for a mattress where the head of the baby will lie We use stockinet bands to tie the feet so that they cannot get through the padded hole on which

One of the few other ways in which these studies can be checked is by the data on growth in length and Figure 32 shows what we have found in the rate of growth in length compared with the findings of other people

The curve for linear growth at 110 I U of vitamin D (average intake of an infant given milk containing 135 I U of vitamin D to the quart) has been corroborated by Dr Icie Macy Hoobler of Detroit and by Rapoport and Stokes of Philadelphia in large outpatient studies of infants given the same type of milk containing 135 units of vitamin D to the quart

The same rate of growth as with the infants receiving 340 I U of vitamin D daily was observed by Hoobler in a group of infants fed 600 I U of vitamin D daily and by us in a group of infants given 800 I U daily Rapoport and Stokes noted the same rate of growth for infants fed 1500 I U daily as for those fed 135 unit milk we have observed definite slowing of growth in in fants fed 1800 or more units of vitamin D daily

We feel that these studies of a different type which corroborate our find ings with a small and frequently studied group of closely housed babies in a winter study show that our findings are sound and can be checked by other methods

PRINCIPLES OF CALCIUM AND PHOSPHORUS METABOLISM

Professor NICOLAYSEN (Oslo) When a calcium and phosphorus bal ance study is performed in man or animal the striking feature is that the calcium and also the phosphorus content of the feces are greatly dependent upon the intake

For a long time it was believed that these minerals were absorbed and then fixed in the body according to demand The surplus was believed to be ex creted to a great extent in the large intestine again It has been proved how ever that the colon has no such function at all and that there is no regulated secretion of calcium into the gut It has also been made clear that the ability to absorb calcium is limited although it may vary considerably

When a diet free of calcium is given the feces still contain considerable quantities of calcium indicating that some of the fecal calcium comes from inside the body I have borrowed the term endogenous for that calcium fraction although obviously no calcium originates within the body

The explanation of the origin of this fraction is as follows Man secretes 8 to 10 liters of digestive juices each day the calcium concentration of the juices is roughly equal to that of the blood serum Thus an adult man can

simple The nurses can make them in a few minutes and so they are made fresh for each period of study

The infant s activity is restricted really only in that he must sleep on his back which is not a common position for infants and so they must be trained. When the infant is about three weeks old he is put on the bed for one night s sleeping The next week he is put on for two full days The third week he goes on for a three day collection and thereafter he will be on collections every alternate week

The 24 hour collection is started from the time the urine is voided and is stopped at exactly 24 hours from that time

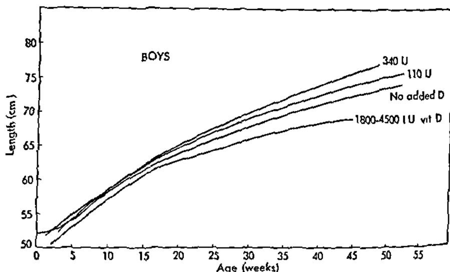


Fig 32 Rates of linear growth of infants given varying amounts of vitamin D daily

With any infection such as a slight cold a child is kept off study for at least two weeks after he has completely recovered Any infection with a real fever lasting a week or more means that the child is then considered not normal from then on and we use the data we have obtained in a study of the effect of and the time which it takes a baby to recover from such an illness Such illnesses occur rarely

The question will at once arise as to whether this method of long study on relatively few infants gives reliable results We believe that it does In our data of creatinine excretion in infants we find a correlation with body weight of 0 9 which is a very good correlation for biological data Using the creatinine as index of skeletal musculature we come to the same findings as Scammon of Minnesota with his anatomical studies Thus our method of study corroborates the findings of an anatomist

ments adequate amounts of phosphate must be fed so that calcium is the one variable

The two most powerful factors in the regulation of the absorption of calcium however are vitamin D and what I some time ago ventured to call the endogenous factor It is my firm conviction that the effect of vitamin D on the absorption of calcium is well established In my own original investigations the evidence in favor of that view was built up in a series of varied experiments excluding some possibilities proving others

What is the action of vitamin D? So far the mechanism remains obscure It may be a direct effect on the epithelial cell or it may be a secondary effect

What is the endogenous factor ? It is well known that the absorption of calcium varies with age However the dominant factor is not the age of the cells but the development of the body as a physiologic machine Experimental animals can be chronically undernourished with calcium and the power to absorb is then kept constant When a parallel group of growing animals is given a diet rich in calcium the absorption will steadily decline to a relatively low level Thus the prehistory with regard to adequacy or inadequacy of calcium in the food in the period of growth is an important factor in determining what amount of calcium will be absorbed from a given test dose at a given time

The foregoing remarks are founded on experiments on rats However observations on children made by Nicholls and Nimalasurya fit very well into this picture On the low-calcium diet customary in Ceylon children absorb nearly every trace of calcium in the diet Thus man and animals to a great extent can economize calcium at least in the period of growth Will they develop optimally under such restrictions? That is a fundamental problem in the feeding of populations and I will go into that later when discussing adaptation

I do not feel sure that I have the full picture of this story and we are still doing experiments to get a fuller description However it should be noted that this endogenous factor described as it is by circumstantial evidence is dependent at least to a great extent on the presence of vitamin D

A number of factors may depress absorption of calcium Oxalic acid is one such depressant Sherman says quite rightly that it was a great mistake to choose spinach for popularization as a typical green leaf vegetable because it is so rich in oxalic acid

Phytic acid is another depressant It may have a great influence on the absorption of calcium which has been demonstrated in different species In other species the effect cannot be observed The combination of a diet containing just the needed amount of calcium and considerable quantities of

secrete nearly 1 gm of calcium into the intestinal tract daily As we all know he may secrete half of his sodium chloride into the intestinal lumen each day However that salt is absorbed with the greatest speed whereas calcium is absorbed slowly And the salts of calcium are readily precipitated at the pH prevailing in the lower part of the small intestine since there is always a surplus in the small intestine of anions forming insoluble calcium salts Thus one has a reasonable explanation why endogenous calcium may be lost in considerable quantities with the feces

Phosphates are absorbed at rather high speed and it appears that when large doses are given man can absorb many times his daily requirement with hardly any extra output of phosphates in the feces The fate of ingested phosphate is entirely governed by the presence or absence of calcium It is a general finding that phosphorus in the feces follows calcium On a calcium free diet some phosphate is still lost with the feces but the quantity depends on the ability to reabsorb digestive juice calcium When that ability is high and fecal calcium very low more phosphorus than calcium may be found in the feces because of the loss of organic phosphorus in the bacteria When the ability to absorb calcium is low the usual picture is slightly more calcium than phosphorus in the feces

It is quite clear that the absorption of calcium from exogenous sources and from the digestive juices should be considered together The intestine cannot distinguish between calcium ions of different origin It is also clear that only dissolved salts can be absorbed what is not in solution will simply pass straight through

What is known about calcium absorption? The absorption may be found to vary between two extremes a nearly complete absorption or an amount of calcium in the feces which equals or exceeds food calcium

Species differ to a certain extent in absorptive power The rat for example absorbs relatively more calcium than any other species I know of which has been studied Not all types of experiments have been done in each species Consequently the picture I can form is a sort of jigsaw puzzle using data obtained in different species for generalization As far as my knowledge goes there is no qualitative species difference The qualitative characteristics observed in one species can be applied to other species as is done in nearly every other special field in physiology and biochemistry

What are the factors influencing the absorption? I have used the isolated loop technique to study these One factor of course is the amount of calcium fed and up to a certain limit the amount absorbed increases with the amount fed although the percentage absorption goes down It seems clear that concentration of calcium is one factor of importance In these experi

turnover of salts in the bones is then to a limited extent only regulated by vitamin D in an unknown way

Citric acid also enters the bone picture It is always lower in vitamin D-deficient rats as compared with parallel animals given vitamin D Studies going on in my department indicate that the metabolic activity of the cartilage is rather high and that the citric acid cycle is involved

A number of other factors influence the metabolism of the bone cells Parathyroid hormone and excess vitamin D cause bone dissolution and sex hormones have been shown especially in birds to accelerate the formation of bone trabeculae to the extent that the marrow cavity is obliterated by newly formed bone

I shall leave out of the discussion how the parathyroid gland influences the bone formation An important point to discuss however is the effect of physiologic and toxic doses of vitamin D

It is quite clear that vitamin D promotes absorption of calcium not only in physiologic doses but also in toxic quantities One thousand units in a rat promotes more calcium absorption from a given amount of calcium than a few units However the effect on the bones is different The small dose will promote calcification but the toxic dose will cause dissolution of bone and will increase the amount of circulating bone salts

When discussing the mode of action of vitamin D this difference between what I prefer to call the physiologic effect of vitamin D as contrasted with the pharmacodynamic effect should be kept clearly in mind especially when the action on the reabsorption of phosphates in the kidney is discussed It has been claimed that vitamin D acts on the reabsorption of phosphates in the tubules However the number of units of vitamin D used was quite high When the balance studies in the literature are scrutinized no evidence can be found in favor of the view that the transition from a vitamin D-free diet to one containing vitamin D is followed by a decreased phosphate excretion in the urine I believe that the problem should be reinvestigated

Before discussing adaptation some remarks on the skeleton are needed The skeleton is built according to a definite morphologic and chemical pattern It is most probable that there is an upper limit to the density of calcification and that this upper limit is the physiologic optimum According to this view there is no depot of calcium and phosphorus in the bones in the sense that glycogen in the liver and subcutaneous fat are depots Any loss of salts from the bones is a real loss

Adaptation to a reduced level of calcium in the food may take place by a reduction in the output of calcium in the urine or by a more efficient utilization of calcium in the intestines In the adult any surplus of calcium brought

phytic acid should be avoided A diet which is very liberal in calcium can to my mind contain a certain amount of phytic acid and no harm is done The clear-cut answer is thus dependent upon a precise definition of the *minimum optimum calcium requirement at different ages* I have found that the effect of phytic acid is much more pronounced when the absorption of calcium is high than when it is low The consequence should be that phytic acid is much more of a problem in the years of growth than later It would fit in well with the tremendous effect observed in Denmark of phytic acid in children

Citric acid has been much under discussion since it was first found to have a certain beneficial effect *on rickets in rats* In certain diets mixtures of sodium citrate and citric acid very definitely promote calcification Citric acid and other hydroxy acids forming complex calcium salts can promote the absorption of calcium However it appears probable that the effect of citrate is limited to diets rich in phytic acid We have just found in my department that no effect results when a citric acid–citrate mixture is included in a rachitic diet free of phytate That is in complete accord with the bone ash studies made by Day ten years ago

Attention should for a moment be drawn to the profound aftereffects of sodium citrate on the loss of calcium in the feces claimed by Mitchell and Steggerda in 1942 In a number of experiments on rats we have tried to *repeat this but with completely negative results It is difficult to understand* why citric acid which is metabolized so quickly in the body should influence the calcium metabolism weeks later

Some years ago it was thought that oleic acid had a beneficial effect on the absorption of calcium that is not so Although calcium absorption is slightly better on a diet containing fat than on a fat free diet it is only in diseases with faulty fat absorption that fatty acids are of any great importance *in the absorption of calcium* The development of tetany and osteomalacia following chronic steatorrhea is well known

Bones are formed by a mechanism far from fully understood and I shall only touch on the problem According to the latest studies in the United States the bone salts are *in a very active equilibrium with the plasma* In vitro studies with isotopic phosphorus demonstrate a 50 per cent turnover in 48 hours in trabecular bone Studies with isotopic calcium in vivo support this view Numerous studies indicate that vitamin D has a direct action on the bones The ability to calcify cartilage or osteoid tissue is not lost in vitamin D deficiency however Bone salts may be laid down in vitamin D-deficient bones in nearly the same amount as in bones in a vitamin D-fed rat Excess trabeculae are formed however and the structure is abnormal The

Arguments from the food habits of primitive people have been used in favor of adaptation However it should be kept in mind that we do not know if these people develop optimally They may retain a high power of absorption because of chronic underfeeding and their skeletons may mature at a later age This most probably is so Nevertheless calcium may be a factor of importance in the speed of growth To my mind Aykroyd s experience in India is very important in this respect He found that the mere addition of calcium lactate to the diet of children increased the rate of growth Calcium can accordingly very well be a factor in stunted growth

It has also been claimed that adaptation could take place within a relatively narrow limit much more difficult to observe Mitchell and Steggerda think it is improbable that persons found to be in negative balance on about 10 mg per kilogram of body weight could not adapt themselves in the course of time to this average intake of calcium It might well be however that different individuals have different inborn characteristics as regards calcium metabolism so that results obtained in one person or a limited group of persons could not be freely applied to others

It might be said that studies on adults are not so very relevant in a discussion of early childhood However a high flexibility in adults would call for less attention in childhood and vice versa

In long term studies on rats which have now been repeated we find in adults subjected to calcium starvation that the ability to absorb calcium remains unaltered for a long time The implication would be that the absorption is not adaptable in the matured body until skeletal damage is present

In other studies we find that we can sort out rats with a continuously higher ability to absorb calcium than other rats Of a group of twelve rats studied in ten months two had continuously the highest ability to absorb and two the lowest

Dr Malm is doing long term studies in adult men So far we have one man of 12 studied who has been in negative balance for nearly a year on between 10 to 11 mg calcium daily per kilogram of body weight

It is of interest in this connection to mention briefly the observations on the urinary output First it appears to be an individual constant under constant experimental conditions and in adults it may vary up to several hundred per cent from person to person The man with the negative balance has continuously belonged to the type with a relatively high urinary excretion Thus in him better economy was not effected either by depression of urinary excretion or by improved absorption

Now when I scrutinize studies of calcium metabolism in childhood as reported in the literature I find some indication that children also may be di

into the blood stream above what is needed for maintenance will be excreted in the urine The growing body apparently stores nearly all calcium absorbed Wide ranges of intake are followed by very small variations in the urine

However when the calcium intake is reduced to a low level urinary calcium goes down In chronic calcium underfeeding in children it may go down to a few milligrams daily Thus the body within certain limits can *economize by a reduction of the urinary output*

Can absorption vary according to demand and supply? The problem is very involved and we do not know the full story In the following I shall discuss what I consider are the most relevant observations and what conclusions I feel can be drawn from them

1 The Growing Body It has been emphasized that young animals fed continuously on a low-calcium diet in the period of growth can keep up a very high rate of absorption When they are fed a diet liberal in calcium the absorption goes down This will happen at a relatively early age if they are fed on a diet rich in calcium It will happen very much later if calcium in the diet is restricted Age then is not the decisive factor but the saturation of the body with calcium It is clear from the already mentioned observations of Nicholls and Nimalasurya that children can behave in essentially the same way I know of no complete lifetime study of this specific problem However it might be useful to recall that Henderson and Kelly found a very low fecal calcium in young adult Negroes whose diet is said to be very low in calcium It is probable then that a continued dietary deficiency of calcium would be associated with a continued high efficiency of absorption

2 The Adult Body The impression obtained from an enormous number of balance studies is that the absorption is low as compared to the absorption in the period of growth In the literature numerous reports are found on calcium balances on a low-calcium diet in presumably normal persons Rose reports studies with approximately 0 25 gm calcium in the day s food and retention of calcium Sherman in his one hundred studies with 0 3 gm calcium per day and the Aub group in Boston with 0 1 gm as the daily intake report considerable loss of calcium In advanced cases of hyperparathyroidism and in osteoporotic adults a very low fecal calcium is observed indicating a highly efficient utilization It appears that adaptation or in other words efficient utilization of the ingested calcium may occur The pertinent problem is Does it recur as soon as calcium underfeeding starts does it need some time and then how long or does it not occur again until the skeleton has suffered a severe loss of calcium as is the case in osteoporosis and hyperparathyroidism?

wide It is good that we are in possession of this gift but it should never be drawn upon if not necessary The evidence in favor of a liberal supply of calcium appears to be so strong the sources of it in nature are so cheap that if necessary one should not hesitate to fortify food with it

In nearly all circumstances it will be calcium and not phosphate that will be the limiting factor Therefore this discussion was chiefly a discussion of the physiology of calcium However when milk is the chief source of food as in infancy and vitamin D is not present the low absorption of calcium will be associated with a low absorption of phosphates In the body the soft tissue will have a higher affinity for phosphates than the bones The bones then may be very little calcified because the soft tissue will take what phosphorus is brought into the blood stream Then phosphate will be the limiting factor in the growth and development of bone tissue

THE FUNCTIONAL RELATIONSHIP BETWEEN THE HYPOPHYSIS AND THE PARATHYROID GLANDS IN CALCIUM AND PHOSPHORUS METABOLISM

Dr TÖRNBLOM (Uppsala) Numerous observations indicate that the pituitary gland and the parathyroids are somehow associated In acromegaly parathyroid hyperplasia or adenomata are common findings Even the combination of acromegaly and osteitis fibrosa generalisata with parathyroid hyperplasia or adenomata has been described

In Cushing s syndrome similar parathyroid changes may also be present but seem to be less common The combination of osteitis fibrosa with basophilic pituitary adenoma has been observed too

Even in cases of hyperparathyroidism the general clinical picture of *which exhibited no signs of pituitary disorder histological changes in the* pituitary gland have been demonstrated In several cases of hyperparathyroidism the Swedish pathologists Mellgren and Wilton recently observed characteristic xanthoma like cells in the anterior pituitary

In 1934 Albright described three cases of osteitis fibrosa with hyperplasia of all the parathyroids Assuming that there must be some factor causing this hyperplasia he reviewed 101 cases of hyperparathyroidism finding that in 17 of these there were multiple parathyroid adenomata or general parathyroid hyperplasia Seven of these cases had been examined post mortem five of these seven had hyperplasia of or adenomata in the suprarenal cortex.

It may be of interest to mention that some of the cases with both pituitary and parathyroid adenomata even had insulomata in the pancreas

The functional relationship between the pituitary gland and the parathyroids

vided into different types in this respect Urinary calcium is found to vary rather considerably within the same age group but comparatively little with intake Children may then just as adults possess individual characteristics as regards urinary output of calcium

A comparison of data accumulated by Icie Macy Hoobler and by Julia Outhouse Holmes is interesting One finds within the same age group that the response to a high calcium intake differs in the two groups Can it be that the prehistory of the children was so different that it can account for the differences seen or do we here have examples of individual differences presumably of endogenous origin?

The problem of adaptation may also be viewed from an entirely different angle It has already been noted that the rat has a remarkable ability to absorb calcium If rats and humans were tested for generations on a diet just sufficient in calcium which species would be likely to show the better adaptability? The reply would undoubtedly be the rat in part because it is the less differentiated species

In the last 25 years a number of studies on rats have been published by Sherman They cover a huge number and generation after generation has been studied The results are very remarkable and I think that these studies require the deepest attention by the student of nutrition

A diet consisting of five parts of whole wheat and one part of whole dry milk has been found adequate to support normal growth health reproduction and lactation generation after generation By 1947 sixty three generations had been produced on this diet It contains 0 20 per cent calcium

However better animals are produced by adding more calcium to the diet The yardsticks in this respect are slightly increased growth earlier maturity higher adult vitality especially as shown in superior breeding records a longer period between the attainment of maturity and the onset of senility and in lesser degree an increase in the average length of adult life

Not only did raising the calcium content of the diet to 0 34 per cent a 70 per cent increase give improved results but still better results were achieved by a further increase in the level of calcium up to 0 6 per cent

Our forecast then would have been entirely wrong The lesson given us by these extremely valuable experiments is in line with what most professional cattle breeders and farmers know To achieve the best economical result they are liberal in the feeding of their animals and they are liberal in manuring the soil

Adaptability is a gift to us from nature It may allow the body to grow and develop and when necessary to economize The margin of safety is rather broad The gap between optimal health and frank disease is rather

plasma calcium remains unchanged after hypophysectomy and the plasma phosphorus does not rise as much

One further point arises from this experiment We do not know whether the increase in weight of the parathyroids occurring when the intact animals are put on a low-calcium high phosphorus diet is due to the reduced plasma calcium or to the increased plasma phosphorus On this diet the plasma phosphorus is lower in hypophysectomized than in the nonoperated animals In the former animals the failure of the parathyroids to gain weight suggests

ANIMALS OBSERVED FOR 48 HOURS

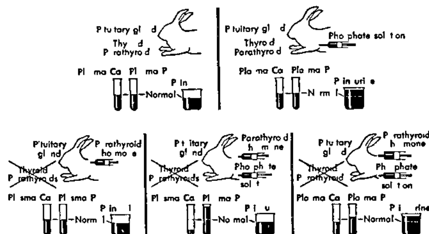


Fig 34 Interrelations of parathyroid hormone plasma calcium and plasma and urinary phosphorus.

that the stimulus to hyperplasia is the high plasma phosphorus rather than the low plasma calcium

These findings may be explained by the assumption that the pituitary gland tends to raise the plasma phosphorus and that an elevated plasma phosphorus induces parathyroid hyperplasia

In order to clear up these problems I carried out some experiments to find out whether parathyroid function is changed by administration of phosphate and if so how soon this change sets in

I have thyroparathyroidectomized rabbits and substituted the parathyroid function by continuous intravenous injection of parathyroid hormone The period of observation was 48 hours the assumption being that the loss of the thyroid function would not become effective in such a short time In the second chart (Fig. 34) it is seen that the dosage of parathyroid hormone was such that a normal plasma calcium plasma phosphorus and urinary phosphorus resulted

has been the object of experimental investigations Atrophy of the para thyroids following hypophysectomy and hyperplasia of the parathyroids following administration of pituitary extract have both been observed Houssay and the German gynecologists Anselmino and Hoffman have discussed these matters Anselmino and Hoffman maintained that administration of pituitary extract is followed not only by parathyroid hyperplasia but also by a raised serum calcium level that is that administration of pituitary extract could induce an increased activity of the parathyroids Anselmino and Hoffman are of the opinion that the pituitary gland produces a parathyrotrophic hormone

Attempting to analyze the connection between the anterior pituitary and the parathyroids I put normal rabbits on a low-calcium high phosphorus diet

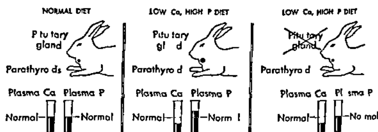


Fig 33 Effect of diet on plasma calcium and phosphorus in the presence and absence of the pituitary gland

(Fig 33) There resulted a fall in plasma calcium and a rise in plasma inorganic phosphorus The parathyroids moreover increased considerably in weight In hypophysectomized rabbits on the same diet the plasma calcium was equally low but the plasma phosphorus was less elevated and the para thyroids were approximately normal in weight

One might conclude from this observation that the failure of the parathyroids to gain in weight in the hypophysectomized animals was due to the loss of a parathyrotrophic function of the pituitary This explanation however is inadequate

The increase in weight of the parathyroids taking place when the animals were put on a low-calcium high phosphorus diet may be regarded as a sign of a compensatory hyperfunction of the glands with the object of counter acting the fall in plasma calcium the rise in plasma phosphorus or both If the failure of the parathyroids to gain weight in the hypophysectomized animals were due to the loss of a direct action of the pituitary gland on the parathyroids it should be attended by a still more marked drop in the plasma calcium and an even greater rise in the plasma phosphorus Actually the

Continuous intravenous phosphate injection on nonoperated animals caused a marked ri e in urinary phosphorus but affected neither the plasma calcium nor the plasma phosphorus When the same amount of phosphate was given to thyroparathyroidectomized animals with parathyroid function replaced by administration of parathyroid hormone the result was quite different In this case there was no corresponding increase in the urinary phosphorus the plasma phosphorus rose and the plasma calcium fell When the animals were given an increased amount of parathyroid hormone the urinary phos phorus and the plasma phosphorus and calcium all reverted toward normal From these experiments I drew the conclusion that administration of phos phate increases the function of the parathyroids and that this change in func tion sets in within 48 hours

As I have said the data shown in my first figure suggested that the pituitary gland increases the plasma phosphorus Now since administration of phos phate rapidly induces an increased activity of the parathyroids we might expect that if the plasma phosphorus level is influenced by the pituitary gland its influence should appear more markedly in parathyroidectomized than in nonparathyroidectomized animals in which compensatory decreased para thyroid activity might obscure a change of the plasma phosphorus

The effect of hypophysectomy on thyroparathyroidectomized animals appears in the third chart (Fig 35) These animals were maintained on a high-calcium low phosphorus diet The increase in plasma phosphorus fol lowing thyroparathyroidectomy failed to appear in simultaneously hypo physectomized animals The next step was to replace the effect of the pituitary gland with administered pituitary extract This caused an increase in plasma phosphorus and reduction in plasma calcium These experiments confirm the earlier conclusion that the pituitary gland increa es the plasma phosphorus Clearly the parathyroids do not transmit this activity

In otherwise intact animals hypophysectomy does not alter the plasma calcium or plasma phosphorus level whereas in thyroparathyroidectomized animals hypophysectomy increases the plasma calcium from lowered to normal levels and decreases plasma phosphorus from raised to normal levels (Fig 35) Thus there is apparently a compensatory decrease of parathyroid function opposing the effect of hypophysectomy This is confirmed by the findings that the effect of a given dose of pituitary extract is more pronounced in hypophysectomized thyroparathyroidectomized than in only hypophysec tomized animals (Fig 36)

In connection with these observations the question arises as to whether the action is due to the anterior or to the posterior pituitary In order to answer that question I shall remind you of the pathoanatomical observations in the

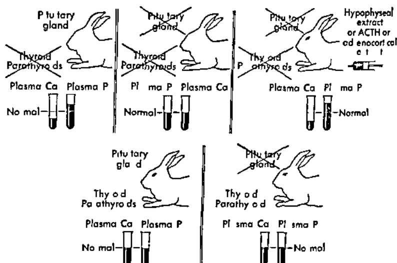


Fig 35 Interrelations of pituitary gland parathyroid glands and calcium and phosphorus

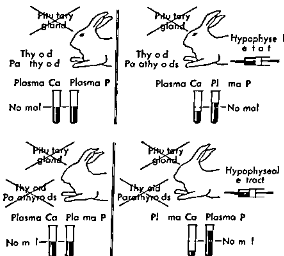


Fig 36 Effect of hypophyseal extract on plasma calcium and phosphorus in hypophysectomized and hypophysectomized thyroparathyroidectomized rabbits

those fluids and therefore I would expect the calcium content of the digestive secretions to be somewhat below that of plasma This is a minor point because one can still get a very large amount of calcium secreted in the intestines

In discussions about racial diet and adaptation over generations to very low intake we must remember that there has been a process of survival of the fit and that probably those who did not adapt well either contracted severe osteomalacia or died The women would die in childbirth Through the ages the selection of those adaptable to the lower intake would go on Further it appears from studies of various people that there has been adaptation in those races also in the type of skeleton The long bones have a small cross section compared to their length and the cortex is very fine The physicians I have met from South India state that this is true and they find a marked difference in the general relative bone cross section length between the Sikhs and the natives of Calcutta

We have confirmed Professor Nicolaysen in regard to citric acid In infants we have found that the addition of citric acid does not improve the calcium retention over that observed in any other curded milk and therefore we considered that the maximum effect of the citrate ion must be achieved by the amount normally present in cow s milk

We agree that the recent history with respect to adequacy of calcium intake greatly affects calcium retention We have observed that the longer a child is relatively malnourished but is not in a state of caloric undernutrition the longer it takes that child to return to an ability to utilize calcium when he is again well fed We have seen young adolescents who took 5 months to get good utilization of a liter of milk containing 400 units of vitamin D In the first month of the study the retention was very poor It increased with each subsequent month reached an approximate maximum at about 5 months and then continued at about that rate Younger children take definitely less time for that adaptation and the few children that we have studied from a poor environment who had reached the age of 15 or more when the epiphyses had closed and the bones were no longer growing in length were not able even after 7 months to achieve the degree of retention of calcium that we observed in the younger girls of 12 to 14 years It seems probable that although retention of minerals can and does continue after bone growth has ceased nevertheless the quantity of mineral retained daily will be relatively low compared to the amounts which can be retained during rapid growth of the long bones

Mitchell has rated 10 mg of calcium per kilogram as a normal adult requirement with 15 mg per kilogram as an ample allowance Summarizing

parathyroids in acromegaly and Cushing s syndrome The action is due to the anterior lobe of the pituitary gland This is confirmed by Engfeldt who has recently shown that extirpation of the posterior lobe does not lower the plasma phosphorus as does hypophysectomy Further Li, Geschwind and Evans have produced an elevation of the serum phosphorus by injection of pure growth hormone

That the influence of the pituitary gland on the parathyroids is not due to a parathyrotrophic hormone is corroborated by the observation of Engfeldt that administration of phosphate induces parathyroid hyperplasia even in hypophysectomized animals

My observations concerning the functional relationship between the pituitary gland and the parathyroids are summarized as follows

The function of the parathyroids is to lower the plasma phosphorus and raise the plasma calcium

A function of the anterior pituitary is to raise the plasma phosphorus and lower the plasma calcium The parathyroids do not transmit this activity

Induced alterations in plasma phosphorus and calcium are met by compensatory parathyroid changes and these changes occur within 48 hours

If induced alterations in plasma phosphorus and calcium are met by compensatory changes of pituitary activity these changes must appear less rapidly or be of lesser magnitude than those produced by the parathyroids

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CALCIUM PHOSPHORUS AND VITAMIN D REQUIREMENTS IN INFANTS I

Professor STEARNS (Iowa City) Up to now it has been my belief that the calcium in various body fluids tended to vary with the protein content of

our own studies of adults and those reported in the literature we made the interesting observation that at intakes of 20 mg per kilogram or above regardless of age no negative balance was found

I would like to use a few figures to elucidate further the effect of the endocrine factors on the urinary excretion of calcium We very fully agree with Professor Nicolaysen that endogenous calcium is excreted in the urine very largely Dr Elisabeth Knapp in our laboratory determined the factors affecting urinary excretion

Figure 37 is a log log chart of the urinary excretion plotted in terms of urinary calcium as per cent of calcium intake on the ordinate with the calcium in milligrams per kilogram daily on the abscissa We had a total of some 600 studies of our own and from the literature We found that the urinary calcium varied with body weight and with the intake but the largest factor affecting urinary calcium was endogenous probably the resultant of the various hormones affecting calcium metabolism

The first two factors are demonstrated in Figure 38 by plotting the urinary calcium as per cent of calcium intake against the calcium intake per kilogram using a log log scale Though the normal range is wide the urinary calcium of any individual subject tends to remain in the same position relative to the mean at any given calcium intake Thus the normality or abnormality of urinary calcium excretion can be determined at any level of intake

In hyperthyroidism and hyperparathyroidism urinary calcium values are always more than three standard deviations above the mean at any given level of intake In hypoparathyroidism the urinary calciums are below the lower limit of the normal In hypothyroidism they are two standard deviations below but not usually three below And in the few studies of pituitary disturbance we could find in the literature wherein urinary calcium was reported we found the urinary calcium tending to be between the second and the third standard deviations above the normal mean But it is not so strikingly variant as in disturbances of the thyroid or parathyroids The amounts of sex hormones are large enough during pregnancy and lactation to cause definite variations At some time during the latter part of pregnancy each woman we studied tended to show a high urinary calcium well above two standard deviations above and often more than three standard deviations above the normal mean In early lactation the urinary calcium tends to be low

We have found urinary calcium a very useful means of determining whether the primary alteration in calcium metabolism is in absorption or is due to loss of calcium from within the body Normal infants excrete amounts of calcium in the urine which are within the normal range for urinary calcium

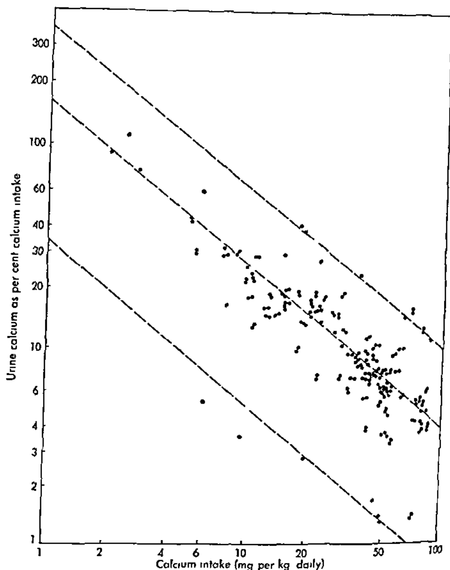


Fig 37 The relationship between urinary calcium expressed as per cent of intake and the calcium intake in milligrams per kilogram daily Subjects are 1 to 80 years of age (Knapp E L *J Clin Investigation* 26 187 1947)

we obtained for children with late rickets of the resistant or refractory type all the values were below three standard deviations from the mean and one child was excreting almost no calcium The arrows and the hollow symbols show the urinary calcium after treatment with vitamin D We use this graph

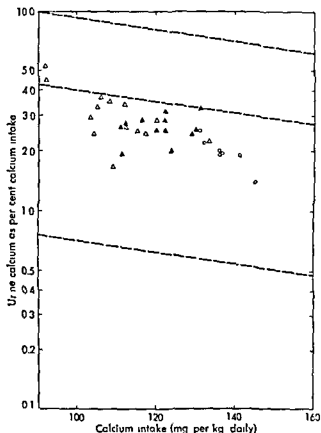


Fig 39 Urinary calcium excretion of infants fed cow's milk in relation to intake In this figure logarithmic scale is used only for the ordinate Values are averaged for each week of age symbols differ for each three months of age with the values for the youngest infants at the right of the diagram (Knapp E. L. *J Clin Investigation* 26 19–, 1947)

as a means of determining the vitamin D dosage necessary for such children We try to keep these children on a dosage of vitamin D which will maintain the urinary calcium approximately at the normal mean for the intake

I would like also to point out that below an intake of 5 mg per kilogram of calcium it is perfectly normal to excrete more than 100 per cent of the diet

but consistently below the normal mean The different symbols from right to left in Figure 39 represent different ages at three month intervals The lower values are shown in children less than 3 months of age and the values finally reach the normal mean between 1 and 2 years of age We find similar very low urinary calcium in adults during recovery from a serious loss

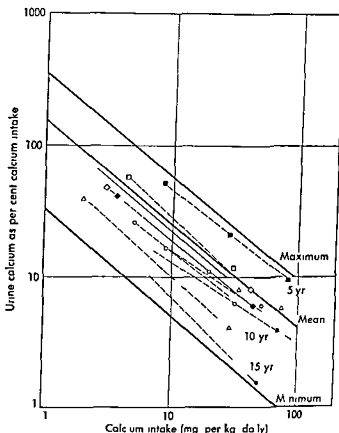


Fig 38 The relationship between urinary calcium expressed as per cent of intake and the calcium intake in milligrams per kilogram daily Urinary calcium of subjects given different levels of calcium intake tends to maintain the same position relative to the mean at all levels of intake

of calcium from bone For example after removal of a parathyroid adenoma one subject excreted only 15 mg of calcium daily for 8 months before the calcium started to rise again Similarly a woman during recovery from a severe osteoporosis showed a calcium excretion of only 8 mg a day a value even lower than those observed in normal infants

We do not have studies of babies with rickets but Figure 40 shows values

large population finds it necessary to adapt to a low-calcium intake it seems probable that those whose endocrine pattern does not fit that type of dietary regimen will not survive over many generations

Professor NICOLAYSEN (Oslo) Perhaps I could say about the calcium content of the digestive juices that my contribution has been a scrutiny of the literature and that I have done no personal study of that problem Fifteen years ago I summarized the analyses I could find in the literature and the variation is very great indeed For example Ball in Boston finds 3 mg per cent in the pancreatic juice and Agren in this country 30 You have to isolate the pancreas and obviously what you get may not be the normal content When I said a rough approximation I mean approximation as a guide to what I think is the essential point namely that considerable amounts of calcium are secreted into the intestines daily with the digestive juice

A very interesting point in Dr Stearns's talk is the sensitivity of the organism to different levels of vitamin D The impression from one type of experiment might be that the physiologic range may be quite large From other experiments it appears quite narrow

We have made a single observation of some interest About a year ago chemical pathologists at a hospital in the neighborhood of Oslo approached me about collaboration on a case of severe osteomalacia of the Milkman type The patient had been in and out of hospitals for 15 years The disease started at 35 years of age We started working on her a year ago I am not going into the details but she had been treated with large doses of vitamin D before and I tried to find a way out from other angles The success was not very striking and so after half a year of study we agreed that we would test her again with high doses of vitamin D I was reluctant to use a very high dose we agreed on 100 000 units daily Now this lady about 50 years old also had a disease of the osteoblasts they could not deposit calcium The blood calcium was normal but when we gave her 100 000 units of vitamin D for two weeks the blood calcium level rose above normal from between 9 and 10 to round about 12 No effect whatsoever on the calcium metabolism was seen and so the dose was reduced to 10 000 units of vitamin D daily * I just mention this to draw attention to the fact that in certain circumstances the skeleton may be much more sensitive to high doses of vitamin D than is the general impression from the literature

Dr CLEMENTS (Geneva) I would like to make an addition to discussions on the apparent inherent variability of individuals with respect to their capacity to utilize calcium I report from work carried out by our unit in

* A repetition of 100 000 units daily in the following year led to an increase in urinary calcium up to 700–800 mg daily

With an intake of 2 mg per kilogram at least half of a normal group will excrete more than 100 per cent of the intake At 2 mg per kilogram intake the upper limit of normal excretion is twice the intake

The normal range of urinary calcium is wide a normal person whose urinary excretion is in the lower part of the range may have only one tenth

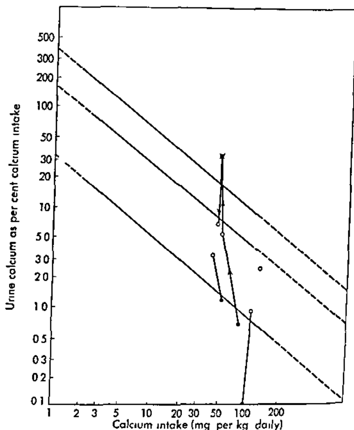


Fig 40 Changes in urinary calcium excretion of children with refractory rickets during therapy with vitamin D Solid symbols indicate values observed before treatment hollow symbols during treatment Lines connect values from a given patient One patient received an excessive amount of vitamin D Urine values returned to the normal mean when dosage was decreased

the urinary calcium of that of a normal person whose urinary excretion is near the top of the normal range Naturally the person whose urinary calcium is low will adapt to a low intake far more readily than will the person whose normal urinary excretion is high Therefore in considering adaptation we have not only the problem of long-continued dietary regimen but also we have the problem of the endocrine pattern in the individual If any

vitamin D–deficient rats was sufficient with 3 per cent calcium carbonate in the diet When rats with vitamin D got 0 5 per cent calcium carbonate in the diet they absorbed approximately the same amount of calcium We had to use subcutaneous phosphate injections in the group with vitamin D deficiency because with a high calcium level in the diet they absorbed hardly any phosphate The net results of these experiments were that the ash content of the bones was found to be identical in the two groups but the structure was entirely different It was even found that we had a higher ash content in some groups without vitamin D than in those with vitamin D but still the structure was abnormal Obviously if you have a bone marrow full of trabeculae as compared with a marrow with hardly any trabeculae and if the ash content is identical in the two bones that means less calcification per unit of bone in the former that is in the group with vitamin D deficiency Vitamin D then acts both on the gut and on the bones It may even be that the effect on the gut is secondary and that the stimulus comes from the bone The terminology is purely descriptive because we do not know how vitamin D acts on the intestinal epithelium

Professor OLSSON (Uppsala) In connection with the use of inorganic and organic acids in feed preservation the question was raised if rickets can be produced in animals fed silage preserved with acids

As a contribution to earlier work in this field four experiments with chicks were carried out at the National Animal Experiment Station Uppsala The experimental plan is given in Table 1

Method Baby chicks were used for the experiments During the first week the chicks were fed a basal (rachitogenic) ration of the following composition ground corn 66 kg soy bean meal 13 kg dried skim milk 10 kg alfalfa meal 7 kg dried yeast 2 kg common salt 1 kg and charcoal 0 5 kg The ration contained 0 33 per cent calcium and 0 43 per cent phosphorus

After the preparatory period the experiments were started The chicks were fed the same basal ration supplemented with bone meal and suboptimal quantities of vitamin D (in cod liver oil) The ration for lot 1 (control in each series) was not supplemented with citric acid The rations to the other groups 2 3 4 and 5 were mixed with varying quantities of crystalline citric acid powder (see Table 1) After 2 weeks the chicks were weighed individually and X ray photographs of their right tarsometatarsal joints were made to determine the degree of bone calcification The degree of calcification was determined by measuring the thickness of the cartilaginous disc (tmt) between the calcification zones of the distal tarsus and the metatarsus according to the method of Olsson The less the thickness of this cartilaginous disc the better is the bone calcification

Australia which was published by Wake in his balance studies of breast fed infants It was apparent from those studies which confirm the work done by Stearns and others on the artificially fed infants that some infants had a capacity to abso b up to 70 per cent of the calcium offered That appeared to be an inherent characteristic of the child and nothing you could do except greatly reducing the calcium intake would affect that figure

More important still was the apparent lower capacity to absorb calcium of infants at the lower end of the scale Some infants had a capacity to absorb only 25 to 30 per cent of the calcium offered under apparently similar conditions of housing diet care and so on No addition of vitamin D affected the percentage of absorption of calcium from those children This I think really confirms the point that has already been made that there is throughout the world this marked variation in the capacity of humans to utilize the calcium offered at various ages

Dr THORELL (Stockholm) Professor Nicolaysen said in his paper that bone salts may be laid down in D deficiency in the same amount as in normal bone tissue As I understand him the calcium absorption in the gut and the level of calcium in the blood are the essential factors in producing the rachitic bone tissue changes

I think however that vitamin deficiency may also play an important role by acting *directly* on the bone tissue cells Cytochemical studies on scorbutic guinea pig dentine cells showed that vitamin C deficiency produced severe alterations in the metabolic processes linked up with the development of the dentine cells I do not want to go into details I refer to the paper together with Wilton in *Acta pathologica et microbiologica Scandinavica* 22 593 1945 In the case of D deficiency we have not yet made any cytochemical analyses but detailed cytological investigations by Wilton show that here also a great part of the bone tissue changes must be explained by a direct effect of the vitamin deficiency on the bone cells (See A Wilton *Tissue Reaction in Bone and Dentine* Henry Kimpton London 1937)

I want to ask Professor Nicolaysen for his opinion of these views

Professor NICOLAYSEN (Oslo) Experiments on the action of vitamin D on the bones were done about ten years ago I may perhaps try to summarize very briefly the most pertinent points in that paper After the effect of vitamin D on calcium absorption had been demonstrated it was not clear if that was the essential function of vitamin D or not Obviously that could not be tested without having animals with and without D but with identical amounts of mineral salts circulating in the blood You would have to give the bone cells optimal amounts of calcium and phosphate with which to work We did that in successive approximations The absorption of calcium in

The conclusions seemed to be that small quantities of citric acid in rations for chicks of the feed composition used in these experiments had an advantageous effect on the bone calcification. Large quantities of citric acid per kilogram of feed had a rachitogenic effect.

Professor NICOLAYSEN (Oslo). The effect of the citric acid here is quite contrary to the general experience.

Professor OLSSON (Oslo). The experiments are not finished and therefore these results must be judged as preliminary. In planned experiments

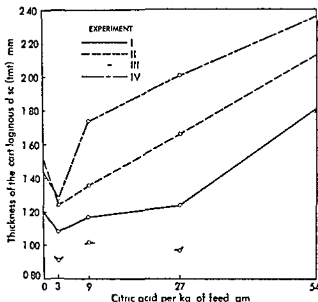


Fig. 41. Effect of citric acid on bone calcification in chicks

I also intend to investigate the retention of citric acid and also of calcium and phosphorus as a consequence of citric acid supplement for chicks.

Professor SIWE (Lund). In these experiments I would only remark that the doses of citric acid are huge, far above the doses that would be normal for chickens. Of course the calcium absorption must be disturbed in such experiments but that does not concern normal metabolism.

Professor OLSSON (Oslo). It is true that the highest doses of citric acid used in these experiments lie above the normal doses used for chickens. The results obtained however have only shown that citric acid, in spite of its organic nature, can produce rickets in chicks. I do not know if this result is

TABLE 1

Results of Feeding Experiments with Citric Acid in Rations for Chicks

Group No	No of chicks	Gm per kg of feed: Cod liver oil*	Gm per kg of feed: Citric acid	At the end of the experimen: Mean weights of chicks gm, Males	Mean weights of chicks gm, Females	Tarsometatarsal cartilage thickness (tmt) in mm (corr for wt)
		Experiment I Started 8 January 1943				
1	20	1 8	—	149	133	1 21
2		1 8	3 0	150	122	1 08
3		1 8	9 0	171	121	1 17
4		1 8	27 0	147	134	1 24
5		1 8	54 0	150	110	1 82
		Experiment II Started 18 January 1943				
1	20	1 8	—	139	114	1 53
2		1 8	3 0	130	117	1 24
3		1 8	9 0	131	109	1 36
4		1 8	27 0	130	114	1 66
5		1 8	54 0	106	94	2 14
		Experiment III Started 4 May 1943				
1	20	0 8	—	108	97	0 98
2		0 8	3 0	110	100	0 91
3		0 8	9 0	119	104	1 07
4		0 8	27 0	123	99	0 97
5		0 8	54 0	123	113	1 80
		Experiment IV Started 7 December 1943				
1	20	1 6	—	140	122	1 44
2		1 6	3 0	141	141	1 29
3		1 6	9 0	134	89	1 74
4		1 6	27 0	130	95	2 01
5		1 6	54 0	123	107	2 36

* Containing 1100 I U vitamin A and 180 Chick Units vitamin D (1 Chick Unit = 0 01 gamma crystalline vitamin D in antirachitic effect) per gram

Results and Conclusions The experimental plans and data are given in Table 1 It was found that the tmt means varied and in consequence also the bone calcification depending on the different supply of citric acid in the rations for the experimental chick lots of each series The bone calcification in the control lots (not supplied with citric acid) was of a lower degree than in lots supplied with 3 gm citric acid per kilogram of feed On the other hand lots supplied with large quantities (27 to 54 gm per kilogram of feed) of citric acid showed a lower degree of bone calcification than lots supplied with 3 gm The results are illustrated in Figure 41

The conclusions seemed to be that small quantities of citric acid in rations for chicks of the feed composition used in these experiments had an advantageous effect on the bone calcification Large quantities of citric acid per kilogram of feed had a rachitogenic effect

Professor NICOLAYSEN (Oslo) The effect of the citric acid here is quite contrary to the general experience

Professor OLSSON (Oslo) The experiments are not finished and therefore these results must be judged as preliminary In planned experiments

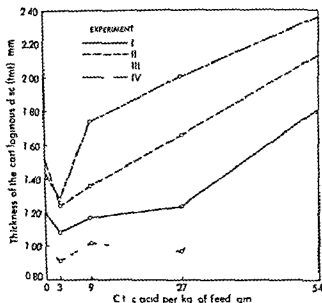


Fig 41 Effect of citric acid on bone calcification in chicks

I also intend to investigate the retention of citric acid and also of calcium and phosphorus as a consequence of citric acid supplement for chicks

Professor SIWE (Lund) In these experiments I would only remark that the doses of citric acid are huge far above the doses that would be normal for chickens Of course the calcium absorption must be disturbed in such experiments but that does not concern normal metabolism

Professor OLSSON (Oslo) *It is true that the highest doses of citric acid* used in these experiments lie above the normal doses used for chickens The results obtained however have only shown that citric acid *in spite of its* organic nature can produce rickets in chicks I do not know if this result is

rachitic animals than in those of normal animals In rickets the alkaline phosphatase in the serum often increases before any other symptoms appear Upon administration of vitamin D the serum phosphatase value gradually returns to normal at the same time the other pathological changes disappear Morris and associates assumed that the bones require abnormally large amounts of phosphatase enzyme when there is a vitamin D deficiency The increase in the serum phosphatase content they take to be secondary and conditioned by enzyme from the skeletal system Vitamin D is necessary for normal ossification beside regulating the absorption and loss of phosphate the vitamin probably also has a direct effect on the skeleton itself It seems possible that the effect of vitamin D on the phosphate metabolism may be due to an effect on alkaline phosphatase in which the changes that are obtained through administration of vitamin D are reactions which can be described by changes in the activity of the alkaline phosphatase Cohn and Greenberg have assumed that vitamin D facilitates the transition from organic to in organic phosphate and can in this way exert a direct influence on ossification

Vitamin D undoubtedly influences the absorption of phosphate from the intestines To a certain extent rats can utilize the phosphate in phytin Their ability to do this declines considerably if they contract rickets but is rapidly restored after administration of vitamin D Harrison and Harrison have been able to show that the administration of vitamin D to rachitic dogs increases the reabsorption of phosphate in the kidney tubules

If the activation of alkaline phosphatase from the kidneys intestines and bones which we have obtained experimentally in vitro after the administration of D P (phosphorylated vitamin D) also occurs in vivo it is possible that this effect explains the changes which are observed after the administration of vitamin D to a rachitic animal The phosphate absorption is improved since the phosphate esters which the intestinal epithelium cannot absorb are more effectively split There may also be a direct effect on the absorption mechanism The utilization of endogenous phosphate becomes more effec tive inasmuch as less is lost in the urine The possibilities for absorption of phosphate by growing bones are improved

Professor NICOLAYSEN (Oslo) It has been very stimulating to listen to Dr Zetterstrom s talk and I think that there is a lot to be said in favor of the view that more attention perhaps should be directed to the phosphate metabolism in the bones than has been done up to now

With regard to absorption of phosphates in my study on the mode of action of vitamin D about 15 years ago the first problem was obviously to find some reasonable explanation of the differences in the fecal excretion of calcium and phosphates as between vitamin D-deficient and normal animals The re

point It may be asked whether the action of vitamin D changed in this way is comparable with the action of unphosphorylated vitamin D in vivo or perhaps whether vitamin D acting in vivo is also phosphorylated

Dr ZETTERSTRÖM (Stockholm) I cannot answer the second question

Professor MELLANDER (Goteborg) Where in the vitamin D molecule do you introduce your phosphoric acid residue?

Dr ZETTERSTRÖM (Stockholm) In the hydroxyl group

Professor MELLANDER (Goteborg) Is this activation effect observed also with other substrates?

Dr ZETTERSTRÖM (Stockholm) If I use other substrates a split of the phosphorylated vitamin can occur and therefore it is not easy to determine the effect

Professor MELLANDER (Goteborg) It has to be stressed that we do not yet know which substances are the physiologic phosphorus carriers in the sense of Robison We know however that different organic phosphoric esters have entirely different sensitivities to the same phosphatase and it is also probable that the pH optimum varies with different substrates

Dr ZETTERSTRÖM (Stockholm) I think there are different phosphatases with different pH optima There are some investigations done by American workers which indicate that the phosphatase preparations consist of different components which can be separated One is active for hexose phosphate and another one is active for glycerophosphate They have shown that the phosphatase which has its pH optimum at one pH is specific for one phosphate compound

Professor BARNETT (New York) I would like to report some observations on the effect of parathormone on phosphate excretion which I think are related to both subjects that we have been discussing this morning This very confused subject on how parathyroid hormone affects phosphate excretion has I think been clarified by some observations of Michie of Philadelphia to be published I think in the *American Journal of Physiology* very shortly In adult human subjects given doses of parathyroid extract as high as 300 or 400 units intravenously he observed that there was a large increase in phosphate excretion which occurred in the absence of any change in glomerular filtration rate Thus in so-called intact adult human subjects his observations seem to demonstrate without question that parathyroid extract does decrease tubular reabsorption of phosphate The interesting fact about this is that if he infuses phosphate and raises serum phosphate to a high level there is a gradual fall in the amount of phosphate reabsorbed by the tubules which reaches a minimum and which is the same as that achieved

sult you may remember was that on a calcium free diet I could find no difference whatsoever in phosphate absorption regardless of what type of phosphorus compound was fed—inorganic phosphate phosphate crystals nucleoprotein phosphate etc In any case when the diet was free of calcium in rachitic animals and we tried to increase the level of phosphate in the diet we would reach a limit when the rats would not eat any more because they got tetany Still they were able to absorb many times the daily requirement Later on when I tested the absorption of phosphate from isolated loops I also found the same speed of absorption with glycerophosphate in vitamin D-deficient rats as in rats given vitamin D

Professor RÄIHÄ (Helsinki) I should like to mention some experiments in Helsinki on rickets in dogs We found that there is a big difference between dogs In some dogs native in Finland for hundreds of years it was very difficult to obtain rickets but in some imported dogs it was very easy There must have been some change in the race during centuries of adaptation

We have some observations on muscles Vitamin D given to a child with rickets will improve the muscular tone very quickly In rachitic dogs we stimulated the muscles electrically to tetanus and observed that such a muscle became fatigued more easily and could do less work than a muscle of a normal dog Furthermore samples of muscles taken during the periods of work from rachitic dogs contained large amounts of phosphate esters This is not true of normal dogs If vitamin D is given to a rachitic dog there is a prompt diminution of the phosphate ester fraction of the working muscle

We also observed that the amount of serum calcium which can be absorbed by barium sulphate when the blood is shaken with barium sulphate is less in the rachitic than in the control animals When vitamin D is given to th rachitic dogs the fraction of the serum calcium absorbable by barium sul phate increases This increase is noted normally after administration of vitamin D but if the dog is immobilized in plaster it will not be seen until the plaster is removed and faradic stimulation applied

We understand that there are two phases of fixation of calcium in bone one is absorption of calcium from the blood and the other is active formation of bone Perhaps there is some connection between muscle work and the absorption of calcium

Professor MELLANDER (Goteborg) The fact mentioned by Dr Zetterstrom that vitamin D activates kidney and other phosphatases is indeed very interesting Similar experiments made by our group about two years ago were negative To get the vitamin in solution we used bile salts and the effect was just a decrease of the phosphatase activity We dropped the problem and now it appears that phosphorylation of the vitamin is the essential

point It may be asked whether the action of vitamin D changed in this way is comparable with the action of unphosphorylated vitamin D in vivo or perhaps whether vitamin D acting in vivo is also phosphorylated

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with maximum doses of parathormone Consequently he believes from these observations that the actual control of phosphate excretion largely is through changes in serum phosphate acting on the parathyroid glands The recent observation that with ACTH phosphate clearances may exceed inulin clearances suggesting secretion of phosphate by the tubules would indicate that there is an action somehow mediated through giving ACTH that can effect tubular transfer of phosphorus beyond that which can be effected by the parathyroid glands

I would like to say one other thing in regard to the effect of phosphate intake on the parathyroid gland In a series of infants in whom histologic examinations of the parathyroid glands were made it was reported by Gardner that infants on cow s milk which has a high phosphate content consistently showed larger parathyroid glands than infants on human milk which I think would fit in with Dr Tornblom s observations on rabbits

Dr TORNBLOM (Uppsala) I would like to point out the similarity between the action of the anterior pituitary on the blood glucose and on the blood inorganic phosphorus The anterior pituitary increases the blood glucose and the blood inorganic phosphorus An increase in the blood glucose stimulates the production of insulin An increase in the blood inorganic phosphorus stimulates the parathyroid activity Insulin decreases the blood glucose Parathyroid hormone decreases the blood inorganic phosphorus

We do not know all the details about the action of the anterior pituitary on the blood glucose We do not know anything about how the anterior pituitary increases the blood inorganic phosphorus

Some observations made by Conn and co workers definitely indicate that some of the substances produced under the influence of the anterior pituitary affecting the carbohydrate metabolism such as compound E of the suprarenal cortex diminish tubular reabsorption of glucose It is very interesting to hear that ACTH (via compound E?) has an opposite action on the way in which the renal tubules handle inorganic phosphorus

CALCIUM PHOSPHORUS, AND VITAMIN D REQUIREMENTS IN INFANTS, II

Professor STEARNS (Iowa City) Having already reported our method of study in general I will go directly to our calcium retention data

Figure 42 shows the retention of calcium by infants fed cow s milk and not given vitamin D At the time Dr Jeans and I began our studies vitamin D was customarily given to infants and we did not feel justified in withholding it

These data therefore are assembled from the older literature including studies by Daniels and Stearns The calcium intake is on the abscissa in milligrams per kilogram daily the calcium retention in milligrams per kilogram daily on the ordinate There is no distinction for age but all babies were under 10 months of age There is a very wide variation from a loss of 30 mg per kilogram to a retention of greater than 60 mg per kilogram There is little if any tendency for retention to have any apparent relation to intake and the mean retention for this group is only 10 mg per kilogram despite a high

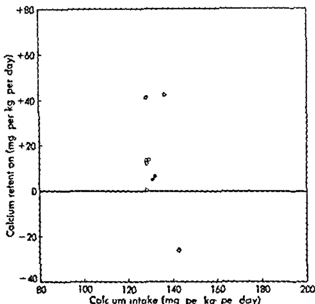


Fig 42 Retention of calcium by healthy infants not given additional vitamin D

mean intake between 130 and 140 mg per kilogram so that although the intake of calcium and phosphorus is high neither is very well utilized by the average baby fed cow s milk without added vitamin D

Figure 43 shows the results of our own studies with infants in a closely housed winter study given milk containing 135 units of vitamin D to the quart This permitted intakes of from 60 to 135 units vitamin D with the average around 90 to 100 units a day *Here a definite relationship between* calcium intake and retention appears The line shows the mean retention for infants given 340 units of vitamin D The calcium retention values for the group given 135 unit milk are somewhat below that of the 340-unit group but the mean value parallels that of the 340-unit group This small amount

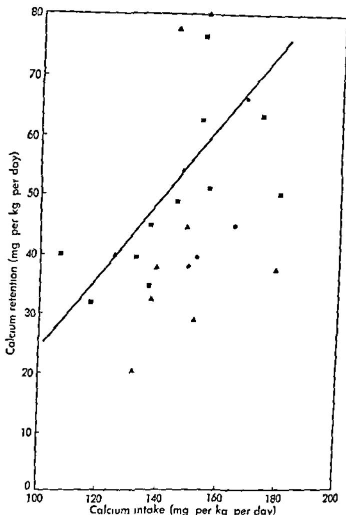


Fig 43 Retention of calcium by closely housed infants given milk containing 135 I U vitamin D to the quart Average vitamin D intake approximates 90 to 100 I U daily (Jeans P C and Stearns G *Proc So Exper Biol & Med* 31 1160 1934)

of vitamin D is sufficient to prevent loss of calcium The minimum retention shown by any baby is higher than the mean retention shown by the group not given vitamin D and the mean intake of calcium is about the same

Figure 44 shows the same sort of study for a much larger group of babies given 340 units of vitamin D daily as cod liver oil or given milk containing 400 international units to the quart Here again we have a very distinct relationship between intake and retention The range of values noted is

rather wide Part of that as I explained earlier is probably due to the short period of study *But again we find that the minimum retention shown by this group is higher than that shown by the preceding group* That is if we add vitamin D to the diet of the artificially fed infants up to this amount, we find fewer and fewer infants with insufficient retention and more with adequate retention Each baby in this group showed fairly wide variations in calcium retention as you would expect *But some babies tended to be consistently close to the minimum others close to the median range a few*

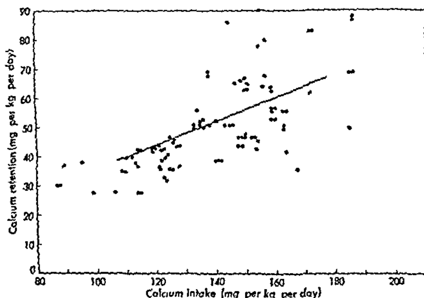


Fig 44 *Retention of calcium by closely housed infants given cow's milk containing 400 I U vitamin D to the quart, or cow's milk plus 340 I U of vitamin D daily as cod liver oil*

tended to be in the upper range and a few in the lower range Giving 800 units of vitamin D a day however we still had about the same distribution of retention Thus with higher vitamin D dosage we did not succeed in having a higher minimum calcium retention nor did we succeed in changing the mean level *When we gave vitamin D in the form of an oily concentrate* which we did in amounts of 270 units 400 units and 800 units we found that by and large the vitamin D seemed to be less effective than it was when more highly dispersed We could find no difference in effect of cod liver oil containing 100 units or less to the gram and of vitamin D dispersed in the milk when the daily dosage in international units was the same

Figure 45 shows the values that we obtained from children fed cod liver oil dispersed in the milk at 1800 units daily or viosterol in approximately twice the dosage and again the mean regression line for retention is the mean regression line for the 340 unit children And we find that these retention values distribute themselves equally on both sides of the line those of the viosterol group are no higher than those of the 1800 unit group

There is, however a very real difference in the mean calcium intake The calcium intake values of all the other groups studied have varied from 135 to

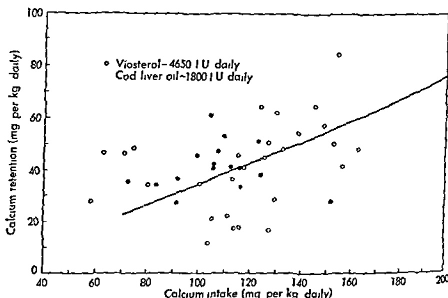


Fig 45 Retention of calcium by closely housed infants given cows milk containing *1800 I U vitamin D dispersed in the feeding or cows milk plus 4650 I U* of vitamin D as concentrate in oil

150 and for the 340 unit babies the mean intake was 145 mg of calcium per kilogram daily The mean intake for this high vitamin D group would lie between 110 and 120 There were 22 infants in this group and 21 of them showed a very distinct drop in appetite by about the fifth month of age What food they took they absorbed and retained but the average intake was very definitely lower than the average intake of those getting 800 units or less

The rate of growth of these four groups of babies was shown in my earlier discussion but I have two figures showing you the rates of growth of some of the individual infants given these high doses of vitamin D In Figure 46 the baby labeled *S* was a small baby to start with Her rate of growth tended to parallel that of the mean range until about 18 weeks of age and then became consistently slower We tried giving thiamin with a transitory effect but at

10 months of age she was definitely a smaller baby in relation to the mean than she had been at the beginning At this time she had to leave us and we asked the mother to give her 350 units daily as cod liver oil We were again able to see her at about 15 months of age and at that time she had made up the difference in growth The baby *J* in the upper curve grew very well better than average until 15 weeks of age and then for 5 weeks showed no growth at all We have come to the conclusion that no baby who is not ill ever stops growing unless there is a specific nutritional abnormality It

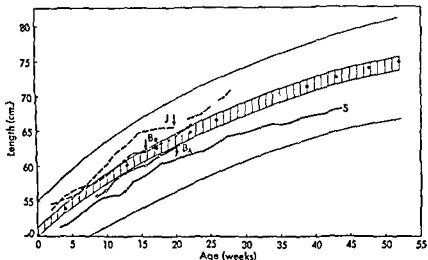


Fig 46. Growth in length of four girl infants fed high dosages of vitamin D compared to Kornfeld standard growth Slowing of growth occurred in babies *S J* and *B* with re-establishment of growth rate by *J* and *B* after vitamin D dosage was decreased Growth in baby *B* had not slowed at 21 weeks (Jeans P C, and Stearns G *J Ped at* 13 735 1938)

may be an overdose or it may be a lack of a certain specific substance But the curve shown by baby *J* was quite typical of the curves that we have seen in these other babies

At the point marked by the arrow all the vitamin D was removed for six weeks with some increase in the rate of growth and then vitamin D at 340 units was started again and his growth was satisfactory for the next six weeks These measurements are taken at two-week intervals so that six weeks with no growth is beyond possible measurement error

Figure 47 shows the rate of growth of baby A who was the only baby not showing any lack of appetite He grew very rapidly in early infancy and had a tremendous appetite at all times His rate of growth was better

the normal and he was the one exception in this group At the point marked by the arrow we dropped the vitamin D to 340 units and as you can see shortly after that period his rate of growth during the next 10 or 12 weeks was at about twice the expected rate Thus even in this one infant who showed no decrease of appetite we found on decreasing the dosage of vitamin D a sharp increase in rate of growth in length over that which was expected at his age

The findings with the 2000 units of vitamin D do not represent acute vitamin D deficiency We had no instance of vomiting The slowing of

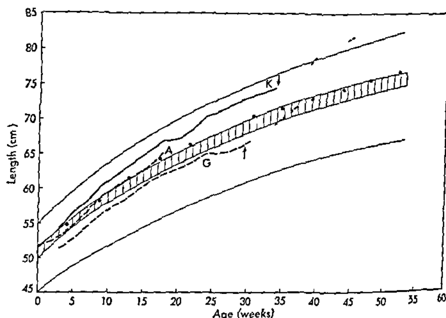


Fig 47 Growth in length of infants given high dosages of vitamin D Baby K received 4650 I U of vitamin D daily and was the only infant of the group whose growth was maintained to 35 weeks of age However when the D dosage was decreased (shown by the arrow) growth proceeded at twice the average rate for the next two months indicating that this baby had grown at less than his normal rate during the period of high dosage Baby *G* showed slowing of growth from 25 weeks with the increased rate after the D dosage was decreased Baby *A* showed no slowing at 18 weeks (Jeans P C and Stearns G *J Pediat* 13 736 1938)

appetite came on rather slowly and most of the infants grew as well as the 340 unit infants up to 20 or 25 weeks of age Then we noticed the specific slowing such as you have seen for baby *J* and by dropping the dosage of vitamin D we secured again a rapid increase in growth

Figure 48 summarizes our findings on the effect of vitamin D on calcium

retention of infants The daily vitamin D intake is shown over the period of infancy both as the mean value per kilogram and as I U daily All these babies were fed undiluted cow s milk feedings suitably curded The upper curve represents retention when it was given in a dispersion of vitamin D of 100 units to the gram The dash line represents retention when the vitamin was fed in concentrated oily sources containing 250 units to the drop The greatest difference in the increase in calcium retention occurs with the first 100 units of vitamin D given The absolute retention is raised from 10 mg per kilogram daily to 40 mg per kilogram daily when 100 I U of vitamin D are given daily With further rise in vitamin D up to 340 units of cod liver oil (or 400 unit milk) we find the maximum retention which is very

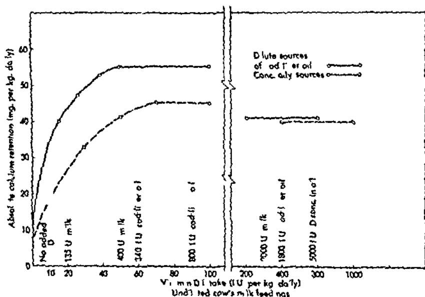


Fig 48 The relation of intake of vitamin D to the retention of calcium in infants

high 55 mg per kilogram With this dosage in concentrated oil medium the calcium retention is 45 mg per kilogram

A calcium retention of 40 mg per kilogram is more than the average breast-fed baby can possibly retain yet the babies who were given cow s milk feeding and retained 40 mg of calcium daily grew at only average rates by Kornfeld s standard and were not superior by clinical judgement Their dentition was definitely slower than that of those infants getting 340 units Several babies of the 100-unit group did not cut any teeth until they were 10 months of age whereas with the 340-unit babies there were very few whom we could keep

to 9 months of age who had not 6 teeth by that time The infants varied very widely in the ages in which eruption of teeth began

There was also a significant difference in the mean muscular achievements of the two groups We kept accurate records of the age at which the infants learned to turn over to sit up to pull themselves to standing to walk with support and to walk alone In each of these the mean value for the 400 unit group was definitely superior to that of the 135 unit group Therefore because all of the criteria tended to give the same result we have felt that 400-unit milk or 300 to 400 I U of vitamin D in a form containing not over 100 units per gram if given in oil promotes the best clinical picture of growth and development in infancy At 800 units we found no measurable improvement in any way over the 340 unit group and so we have felt that it was not economical to go higher We find that mothers have that tendency anyway feeling that if a little is good more is better and so it is our aim to keep the doses down to moderate levels

With the higher dosages 2000 unit milk or 4600 I U as viosterol gave identical results And the actual mean calcium retention in milligrams per kilogram was identical with that of the group getting 135 unit milk only

Because of the decreased rate of growth and loss of appetite we have considered that an amount of vitamin D approximating 2000 units or more in a dispersible form leads to delayed symptoms of chronic toxicity in young infants From the data quoted by Rapoport and Stokes on growth in length wherein they observed the same growth in length with 1500 units as they had observed with 135 unit milk it seems quite possible that this toxic effect may appear even below an intake of 1800 units

Figure 49 shows the quantity of calcium in per cent of total body weight during fetal life and after birth While the rate of increment of calcium is very much sharper in the last months of gestation if it is figured as a percentage of body weight we have a straight line with a definite slope and the mean calcium is 0 8 per cent of the body weight at birth

No matter how a child is fed or how early vitamin D is given we have not been able to get calcium retention in the first eight weeks of life sufficient to maintain the content of calcium present at birth That is true both for infants fed human milk and for those given cow s milk The curves for mean body content of calcium are in percentage of body weight for the cow s milk–fed babies tend to parallel the curves for the deposition of calcium during fetal life The baby who is fed only human milk and who gets a third to a fourth of the amount of calcium of the baby fed on undiluted cow s milk is not able to regain his birth percentage of calcium if one can judge from the figures in the literature Both groups are clinically good infants if the mother s diet

is good But strangely enough the cow's milk-fed infant whose retention or whose percentage of calcium is a little above that of the breast fed infant such as the baby fed 135 unit milk is not clinically as good a baby as the breast fed infant We have concluded that in feeding a baby artificially it is better to allow an ample intake than to try to imitate the intake from human milk because one cannot imitate all of the factors in human milk and in

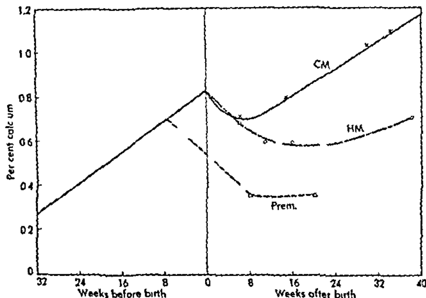


Fig 49 Changes in relative calcium content of the fetus and infant The regression line of calcium content of fetus is drawn from data in the literature *CM* infants fed cow's milk *HM* infants fed human milk *Prem* prematurely born infants fed human milk (Stearns, G *Phys l Rev* 19 416 1939)

evitably the baby seems to be at a disadvantage Dr Levine will take up the problem of the premature infant

We have not studied the use of massive-dose therapy It is not necessary for us in Iowa to feed massive doses of vitamin D Iowa mothers are now trained to give their babies moderate amounts of vitamin D daily and we wish them to keep giving it through the whole period of growth

CALCIUM AND PHOSPHORUS ABSORPTION

Professor SIWE (Lund) Fifteen years ago we began to give just undiluted cow's milk to all babies even the premature ones with or without

human milk as additional food and from that time we have had no signs of early rachitis Vitamin D was of course given, as before from the age of one month I have searched for signs of rachitis in the blood and in the X ray pictures but have found none and I have understood the findings thus that while human milk contains only just as much calcium as is required for the normal full term baby the content in the breast milk is not sufficient to give the premature children what they need Even many full term babies cannot get their need of calcium covered by breast milk If they are fed only human milk they may develop a decalcification of the bones which can easily be recognized in craniotabes This decalcification (craniotabes) will sometimes disappear with larger doses of vitamin D But in many instances even that therapy will not suffice Only by giving calcium in a fairly large dose with or without vitamin D will the craniotabes disappear and normal calcification of the bones set in I wonder if many of the cases of early rickets described in the literature are not of this kind and not true vitamin D deficiency

How large an amount of calcium then, is the minimum required for new born premature infants and full term babies to give normal calcification of the skeleton? For 20 years we have studied the problem and I shall give a short survey of the results Our first experiments were on rats and pigs to learn how much of the calcium contained in the food was retained

The experimental animals were taken in groups from 2 to 3 litters half of them being controls The experimental periods were 3 to 4 weeks after a period of balance The results were analyzed and treated mathematically according to the method of Professor A Westerlund

We found the following

1 If the calcium intake is raised the retention is raised absolutely but is diminished as a percentage

2 The net correlation coefficient between intake and excretion of calcium in diets where K Na is varied is + 0 45

3 If more fat is added to the standard diet the absorbed calcium will increase but probably only to a certain optimum

4 There is a definite influence on calcium retention if magnesium and silica are varied in the diet

5 With increasing age more calcium will be retained

6 Certain organic acids have an influence on the retention of calcium Thus oxalic acid will diminish absorption while citric acid at least in moderate doses will increase it

But what happens if the Ca P ratio is varied? Our feeding experiments on animals gave no clear results perhaps because we had chosen wrong limits of variation The question still remains and has new interest since the prob-

lem of calcium and phosphorus rachitis has been raised Are there two different forms of rachitis or not? That there is an intimate correlation between calcium and phosphorus in the body is obvious If the phosphorus level is raised in the blood the calcium level will sink even below the convulsion limit We see that sometimes when we give vitamin D in therapeutic doses to spasmophilics without giving them calcium at the same time preventively In India in a certain region there is a widespread occurrence of rickets in spite of the sunshine the diet contains huge amounts of phosphorus and is very low in calcium

Just to see how great an effect a disproportion in the calcium and phosphorus intake would cause I made an experiment with rats Two series received five times as much calcium as the controls and two received five times as much phosphorus What happened? In the control series nothing at all of course In the other four series after two to three weeks the animals developed a very pronounced rachitis which could be observed histologically as well as by the X ray In two series I followed the chemical changes in the blood during the two to three weeks and found that in the series given high calcium doses the blood level of calcium in the beginning rose while the level of phosphorus sank But after 12 days or so the calcium level sank while the phosphorus level rose both being below the normal values after three weeks In the series given high phosphorus but normal calcium in the food the blood phosphorus rose in the beginning and fell in the second week while calcium sank in the first week to rise toward the third week both values after three weeks establishing themselves below the normal

Of course it may be said that differences like these in the diets are not physiological Still the experience from that district in India shows that very large differences may exist in different parts of the world

In these experiments on animals vitamin D was not given but the animals were kept in daylight

The problems of *the action of vitamin D on the retention of calcium and phosphorus under different circumstances and the development of rachitis* remain unsolved In experiments on chickens however we have learned that if the cal ium and phosphorus intake is low more vitamin D has to be given to prevent rickets and if the intake of calcium and phosphorus is high a lower dose of vitamin D will suffice This is confirmed by the experiences of babies on different diets (diluted and undiluted milk) The ratio Ca P in the diet seems to be of less importance than the actual content of these substances

However animals seem to be able to adjust themselves to the calcium

content of the food conserving relatively more when the supply is limited Investigations on the nutrition of certain populations in the United States England and Scotland and in other countries seem to show the same for man Certainly there is a minimum for the calcium intake But that varies with many other constituents of the food It varies individually too and in the same individual it varies with the time of adaptation It is impossible to state the optimum except for a certain age rate of growth diet season and individual

Nor is it possible with the wide capacity of our organism to adapt itself to the level of intake of calcium to speak of a calcium minimum with which the organism can maintain a balance between intake and output unless a balance has been achieved after a long period perhaps many months of very small intake But as with the nitrogen minimum so also with calcium it may not be wise to strain the organism s ability of adjustment to the extreme over a long time

Professor NICOLAYSEN (Oslo) What interests me very much is the comparatively narrow limits between what we might call the physiologic optimal dose and the toxic dose of vitamin D I think the data produced are very impressive and it is a warning to people who advocate very liberal feed ing that the amount has to be very closely watched

It is good to see definite data on cow s milk compared with human milk There have been numerous reports in the literature that the calcium absorption was very low from cow s milk as compared to human milk I have often wondered whether—and am very glad to have it cleared up—calcium reten tion is simply a question of the dose of vitamin D That certainly simplifies that problem a lot

It was a surprise to hear that sodium and potassium ions have a definite effect on the absorption of calcium I presume they were given in the form of chlorides I should like to hear more about it and about the details of the extent to which they affect the absorption

Dr ROTHE MEYER (Copenhagen) I was impressed and astonished to hear the border line for toxic doses which you put at about 2000 units In the last few years in Denmark there have been several cases and I remember that in a few of them we did wonder what had happened because as far as we could reconstruct the dosage it had not been very excessive In one of the cases one could calculate the dosage given as between 1500 and 2500 units a day for a period of about 6 weeks and in that case quite obvious symptoms had developed

Some years ago I worked with the massive-dosage vitamin D treatment of tetany and left out preventive calcium therapy No convulsions appeared

After the massive dose there was a rise of calcium within the first 24 to 48 hours to normal and even to hypernormal levels while the phosphorus rose more slowly also to normal or even hypernormal levels within the first weeks

In the reported cases of convulsions which I have been able to find the convulsions appeared within the first 24 hours As the rise of calcium may not occur for 24 to 36 hours they all occur within the interval when one cannot be sure of having obtained a normal calcium level So I am still not sure whether with this treatment of tetany preventive calcium treatment is necessary

Professor WALLGREN (Stockholm) When we used the lower doses of vitamin D we used calcium prophylactically to avoid these tetanic convulsions but since we introduced massive doses of vitamin D we do not use calcium therapy and we have never seen any untoward reactions

Professor SIWE (Lund) My experiences with vitamin D giving convulsions in *tetanic* children if not preventively combined with calcium were not based on the usual preventive doses of vitamin D to healthy children of course

To Professor Nicolaysen I should like to reply that we made these experiments which he referred to in such a way that the ratio of potassium to sodium was changed and we found that there is a net co-variation between the K Na ratio and the retention of calcium with a coefficient that is +0 45 This co-variation is as great or even greater with fat So I do not think there is any possibility of avoiding drawing a conclusion such as I did Thorline in his investigations on the potassium balance found quite the same as an accessory result

Dr CLEMENTS (Geneva) In our laboratory the range of calcium retention extended from 30 to 70 per cent of intake with a mean around 45 per cent and of course the intake varied with the age of the child The criteria of satisfactory nutrition were growth in weight and growth in length The infants were not given extra vitamin D beyond that present in human milk and the amount they obtained by sun exposure Each infant was exposed to sunlight to one half of its body for one hour a day at latitude 36° South in the wintertime between 11 and 12 A M and in summertime between 9 and 10 A M

After from 3 to 7 balance studies on the same infant the infant was then given by mouth 800 international units of vitamin D as a concentrate There was no change in the percentage retention of calcium from that observed before the administration of vitamin D

Our interpretation of these data was that the infant had obtained enough vitamin D by sun exposure under those conditions But I stress the latitude

Professor LEVINE (New York) I have some observations made by my colleagues Gordon and Benjamin which I think might contribute to the understanding of the utilization of calcium and phosphorus from human and cow s milk

Figure 50 shows the retention of calcium on different intakes of calcium when premature infants received human and cow s milk respectively

This shows a group of premature infants fed human milk with intakes of calcium of 56 mg per kilogram and the effect on calcium retention of a three fold increase in calcium intake in the form of cow s milk You will notice that the retention of calcium rose from 27 mg to 114 mg per kilogram It

FIGURE 50

Average Calcium Retention of Young Infants

Infants	Diet*	Calcium Balance: Intake mg /kg	Retention mg /kg	Retention Per cent of Intake	Retention Weight Gain gm /kg
Premature (1 6–2 0 kg)	H M	56	27	45 (33–51)	1 9
Premature (2 4–2 6 kg)	C M	165	114	71 (55–80)	9 4
Full term (Lit)	C M	179†	56†	31† (13–46)	8 9‡

* Supplemented by vitamin D
† Jeans et al
‡ Swanson

is of interest to note that similar intakes of calcium by full term infants in the form of cow s milk resulted in the retention of only one half of the calcium retained by the premature infant One might perhaps explain this discrepancy by reference to the increased growth impulse of premature infants and to their defective intrauterine storage which allows the organism to take up the calcium offered with greater rapidity

Figure 51 shows similar figures for phosphorus retention When the phosphorus was increased fourfold in the form of cow s milk the retention was about three times that obtained on the lower phosphorus intake in the form of human milk Here again one the difference in the magnitude of retention in the and th ant receiving similar intakes

of phosphorus. The evidence from these observations seems to indicate that not only full term infants but also premature infants can retain calcium and phosphorus whether given in the form of human or cow's milk and that they can retain the phosphorus and calcium at higher levels when the intake is increased even when given in the form of cow's milk.

Figure 52 is the curve that Professor Stearns showed. She pointed out that at birth the infant has about 8 gm. of calcium per kilogram of body weight and that in utero the fetus has a much lower calcium concentration.

FIGURE 51

Average Phosphorus Retention of Young Infants

Phosphorus Balance

Infants	Diet*	Intake mg./kg.	Retention mg./kg.	Retention Per cent of Intake	Ca/P	Fraction of Ca Intake Retained Per Cent
Premature (1.6–2.0 kg.)	H M	28	24	87 (84–91)	1.1	45
Premature (2.4–2.6 kg.)	C M	109	63	61 (51–70)	1.8	71
Full term (Lit. †)	C M	139	35	26 (16–31)	1.5	31

* Supplemented by vitamin D
† Jeans et al.

Only in the last two months of pregnancy does the fetus accumulate calcium rapidly.

In these observations premature infants were fed human and cow's milk containing different contents of calcium and their calcium retentions were determined. One notes that the calcium retained when the premature infants were given human milk was far below the level which would be necessary to maintain a calcium concentration in the bones of 8 gm. per kilogram. When infants were given cow's milk with a higher calcium intake the retained calcium rapidly approached the level which is present postnatally in full term infants, namely 8 gm. per kilogram. From this point of view it would seem that a higher calcium intake than can be given with unmodified human milk is preferable for the premature infant.

All of these infants received vitamin D and I want to ask Professor Stearns what she thinks the optimal dose of vitamin D should be for the premature

infant We gave 3000 international units daily we think that perhaps this dosage is too high but that 400 units may be too low We believe however that the rickets seen in premature infants is not primarily a vitamin D-deficiency rickets but a low-calcium rickets due to diminished fetal storage

Professor STEARNS (Iowa City) We have not had the opportunity to study babies born as prematurely as those you have studied We have studied, however several babies who were between 2000 and 2500 gm

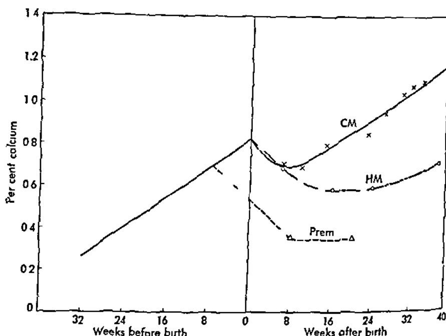


Fig 9 Changes in relative calcium content of the fetus and infant The regression line of calcium content of fetus is drawn from data in the literature *CM* infants fed cows milk *HM* infants fed human milk *Prem* prematurely born infants fed human milk (Stearns G *Physiol R v* 19 416 1939)

at birth and were given 350 or 400 units of vitamin D daily They have not developed rickets and their calcium retention values have been equal to those of the other babies We have used that dosage with all the infants in our premature nursery and have never given more than 400 or 500 units We have not had rickets develop insofar as we know in any of these babies We have considered that they need vitamin D but of course the primary lack is one of calcium and phosphorus rather than of the vitamin

Professor WALLGREN (Stockholm) In discussing vitamin D we should not forget the sunshine and that it may be different in amount in New

York and Iowa City Certainly there are differences from your country to our country Here we have given massive doses of vitamin D to our premature babies and we give human milk but although we give 500 000 units of vitamin D we get rickets in these very small babies of about 1500 gm I believe too that calcium may be a factor that is very important and this is especially true about the smallest premature babies The more we approach the full term the more the vitamin plays the most important role

Dr CLEMENTS (Geneva) We had five pairs of premature infants matched by weight One of each pair was fed cow s milk and one was fed human milk Both pairs were given 400 units of vitamin D in addition to what they got by sunlight I would like to stress that because of my previous observation about sunlight This particular study was quite separate from the other one The sun exposure that these premature infants got was the sun exposure that they would be given either in hospital or after they left hospital with their mothers We had no guarantee as to the amount given but all had tanned a little so that they must have had an appreciable amount of sun exposure

All five infants fed human milk got rickets The infants fed cow s milk did not get clinical rickets though one of them got radiographic changes

Professor NICOLAYSEN (Oslo) I think it might be useful to introduce into the discussion the question of what is rickets? What do different people mean by rickets? I might perhaps start off My view is that the definition must be a temporary one because only if we knew the whole story of the physiology of vitamin D would we be able to give a clear-cut definition As far as I see it the temporary definition might run like this rickets is a disease identical to vitamin D–deficiency disease It is a disease of the bones associated with a metabolic disturbance primarily in calcium metabolism

Professor WALLGREN (Stockholm) I suppose everybody agrees with you that rickets is a deficiency in vitamin D When I talk about rickets in these premature babies as in the case of Dr Clements there was no clinical rickets but there was radiological rickets and this was of course a picture not quite similar to ordinary rickets It was more like osteoporosis But there were still changes in the ulna that are typical for rickets

Dr CLEMENTS (Geneva) I am not so very happy about this definition that rickets is identical with vitamin D deficiency I have collected information over long term studies which suggests that the fetus during its development lays down calcium against its postnatal requirements and that the amount of calcium it lays down depends on the opportunity it has for collecting

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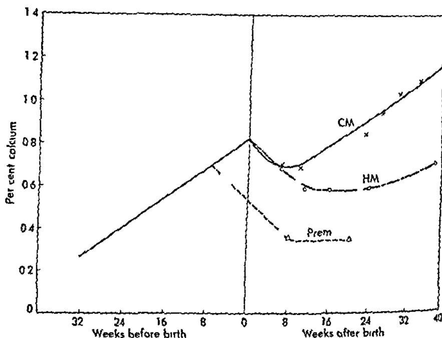


Fig 32 Changes in relative calcium content of the fetus and infant The regression line of calcium content of fetus is drawn from data in the literature *CM* infants fed cow's milk *HM* infants fed human milk *Prem* prematurely born infants fed human milk (*Stearns G Physiol Rev* 19 416 1939)

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calcium There may not necessarily be a vitamin D deficiency It may be a *simple supply problem on the mother s side in that the mother is not supplying* the fetus with enough calcium The infant that has not got 8 gm per kilogram of body weight has much less calcium to draw on for the calcification of new bone produced after birth That being the case it seems to me that the osteate bone laid down during the fourth and fifth month postnatally is inadequately supplied with calcium and is the kind of bone which shows radiographically decalcification of the metaphysis going on to that semi osteoporotic condition that you referred to

Our observations on breast fed infants in the study I referred to showed that it did not matter how much vitamin D that infant got The infant went on progressively getting increasing decalcification of the long bones until about 5 or 6 months of age when it recovered and the calcification was increased The picture in the bones bore no relation to the percentage retention of calcium or the absolute retention of calcium from maternal milk and I could only assume that it bore a relationship to the stores that the infant brought over

Now I do not know whether that is a vitamin D deficiency I looked upon that as Professor Levine does as a calcium deficiency in the stores of the infant

Professor STEARNS (Iowa City) I would like to point out again that all our babies have been studied closely housed No baby during the period of study was exposed to direct sunlight And we have included Negro Italian and Chinese infants in the group We find no difference

After the study was over in the spring we put each group of children out of doors for a couple of months to see if there was a change in retention Those getting 300 to 400 units whether Negro or white showed no change in retention nor in rate of growth The phosphorus retention of the groups tended to vary the mean calcium to phosphorus ratio was about 1 7 with a variation of 1 5 to 2 which is a good retention for the amount of protein and the amount of calcium retained

When we had finished the first three studies we sent all of the X rays to Dr Martha Elliot for her reading They came back with an astounding result *Of the 135 unit group who had grown slowly none of the infants showed* any degree of rickets as read by Dr Martha Elliot But of the rapidly growing infants in the 340 unit group each infant at about 3 to 4 months of age showed what Dr Elliot then termed B 1 rickets that is the very first and faintest degree of rickets which is *a slight roughening of the line* This finding precipitated discussion in pediatric circles in America

We had serum calcium and phosphorus levels of each of the babies during

the period they were supposed to have had rickets and all were normal Their retention values were such that it could not be possible that rickets had anything to do with calcium and phosphorus deposition in bone The final consensus of the group that it was sent to including Dr Elliot was that in judging the fine degree of rickets which she had called B 1 rickets you must know the history of the infant If the feeding history was poor the probability was very great that this was the beginning of true rickets If the feeding history was very good and the infant was growing rapidly then it was only a slight roughening due to the rapidity of growth In none of these instances did the process go any further It usually was visible in two consecutive films and then smoothed out again

Apparently very rapid growth with ample retention of calcium and phosphorus can result in a bone growing too rapidly with deposition too rapid to be quite smooth

Professor SIWE (Lund) You can define rachitis pathologically and anatomically and it is not to be confused with osteoporosis or osteomalacia You can define it clinically and etiologically Clinically it depends upon what methods you find to be the most sensible Personally I do not hold the X ray examination for the most sensible one I am of the opinion that rachitis is a disturbance of not merely calcium and phosphorus metabolism it is a *general* disturbance of metabolism We are used to judging the degree by determining calcium and phosphorus only because that is the easiest way for us to learn how much the metabolism is disturbed Still I quite agree with Professor Nicolaysen We ought to define rachitis as a vitamin D deficiency always bearing in mind that similar changes in the bones in experimental animals for instance rats may be produced by calcium deficiency or phosphorus deficiency I should like to stress that the rapidity of growth of course is a very important factor in making this disturbance of the metabolism visible We see very often that at different ages different parts of the skeleton show rachitical changes in very different degrees

Professor WALLGREN (Stockholm) Do I understand Professor Nicolaysen rightly that rickets is a deficiency in vitamin D and a disease of bones? I would change that to say that rickets is a disease of the whole body with some signs in the bones Deficient absorption of calcium is perhaps one of the signs of rickets as is also the muscle weakness I wonder if it is possible clinically to differentiate between osteoporosis due to calcium deficiency and true rickets due to vitamin D deficiency

Dr RANSTROM (Sundsvall) No it is not possible

Professo LEVINE (New York) Does osteoporosis appear in growing bone? It seems to me that you have to differentiate between osteoporosis

in a bone that is already grown and osteoporosis in a growing bone that may give the picture of roentgenographic rickets I think the presence of growth plays an important part in the definition I do not think rickets occurs in an adult and I think it is rare to see pure osteoporosis in an infant

Professor BESSEY (Chicago) It seems to me that the fundamental process in osteomalacia and rickets is the same In rickets attention is drawn to the growing ends of bones because that is the site of the principal lesion in a growing animal However in a growing young animal there is also delayed calcification of osteoid which is a picture similar to that in osteomalacia But attention is primarily attracted to the growing bone in rickets because changes are so conspicuous there It seems to me the best definition that we can give at present would be a pathological one In rickets the rate of growth and differentiation of cartilage in the epiphysial region goes on normally but the cartilage does not mature and degenerate at the same rate as in normal animals because of either a lack of calcification or a lack of that function of vitamin D which has to do with the maturing of cartilage

Professor NICOLAYSEN (Oslo) I do not think we are in a position to give a clear cut definition but only a temporary one I think we may say rickets is a disease of the whole body and of metabolism but obviously not of every process of metabolism We have to discover what in the metabolism *is* affected and when we know that we have to determine whether this is a primary or secondary effect This is a hypothetical discussion because we have not the knowledge to give a reply

I do not think there is any contradiction between my view and that of Dr Clements I tried to emphasize that when I said that one could clearly distinguish between quantitative and qualitative differences *It is quite clear* that if you have a bone which is growing and you give it vitamin D and no calcium it cannot calcify But I think it is plain that calcium must be provided whether vitamin D is acting on the bones or not

Dr ROTHE MEYER (Copenhagen) We have learned that Dr Levine is accustomed to give 3000 units of vitamin D daily to premature infants and that Dr Jeans would advocate 400 units What then are we to do and where should we settle at least experimentally? Dr Levine when do you start and for how long a time do you give an increased dosage of vitamin D? Should dosage be adapted to rapid growth of the premature or should it be adapted to the birth weight of the premature? It is quite possible that a dosage which may be assimilated by a premature of 2000 gm will not be used efficiently by a premature of 1000 or 1200 gm

Professor LEVINE (New York) If it is true that vitamin D acts by facilitating the utilization of calcium and if it is true that all you need is 400

units to give maximal utilization of calcium then theoretically that should be an optimum dosage Since Professor Stearns showed that one of the best criteria of optimal utilization of vitamin D was not necessarily calcium retention but growth in height I think one might as we plan to do now give 500 to 1000 units of vitamin D to premature infants and compare the rate of growth in height with the infants we have been studying on 3000 units and whose height we have for the last 10 or 15 years If and when those data become available one might be able to say in a clinical sense that 400 500 or 1000 units might be as effective or more effective than 3000 units From the physiologic point of view I should say that the administration of more calcium than is contained in human milk is more important than the absolute amount of vitamin D above a minimum level in premature infants irrespective of birth weight And the lower the birth weight is the more calcium is necessary

Dr von SYDOW (Sundsvall) It has been generally agreed that deficiencies of both minerals and vitamin D during fetal life have been recognized as possible causes of rickets in infancy but it has hitherto been generally considered that these factors do not play any part in causing rickets in the full term infants of mothers who are not suffering from osteomalacia

It is however known that there is a wide variation in the mineral reserves even of full term infants One cannot rule out the possibility that the body of the mother may be severely depleted of minerals especially after repeated pregnancies and lactations accompanied by a low intake of milk products She is obviously likely to suffer from deficiency of vitamin D as well if work illness or climate prevents her from exposing herself to sunlight and if she does not take vitamin D Although it is held that this will not affect the development of the fetal skeleton unless the mother herself develops a manifest osteomalacia many observations have been made which suggest that this is not the case

It was thus no surprise when Follis Jackson and Park in Baltimore presented an investigation in which they demonstrated histologically that rachitic changes are common at a much earlier age than had been previously supposed They found them in no less than 21 per cent of all cases examined during the first month of life The youngest rachitic child in this series was 10 days old This in itself suggests that rickets cannot wholly be due to processes starting after birth and this conclusion is supported by a study recently made by Ranstrom and me

In this study we examined the costochondral junctions of all the infants dying in the maternity department of the Sahlgren Hospital in Goteborg during a period The series was subdivided on the basis of the histological

findings The positive group includes only cases which show definite rachitic changes while the negative group includes both normal and doubtful cases The number of positive cases must therefore be considered as a minimal figure Table 2 shows the age incidence

It will be seen that 57 per cent of the total group show unmistakable signs of rickets The incidence increases sharply with increasing age and if one includes only those which survived for at least 2 days it is found that no less than 83 per cent have definite rickets During the very first days of life the incidence is considerably lower but it is of particular interest that even of those dying during the first two days of life no less than 35 per cent show rachitic changes I should like to demonstrate this point with some microphotographs all derived from infants dying within 24 hours of birth

TABLE 2

Incidence of Rickets in Different Age Groups*

Age in days	0	1	2–6	7–15	16–	Total
No definite rickets	49	15	7	3	4	78
Definite rachitic changes	20	15	23	23	22	103
Per cent	35 4			83 0		56 9
Total	69	30	30	26	26	181

* Ranstrom S and von Sydow G Rickets in Newborn Infants *Pediatrics* 4 409 1949 Charles C Thomas Publisher

Figure 53 shows a normal costochondral junction The zone of ossification is straight narrow, and distinct The structure of the adjacent bone and cartilage is normal Figure 54 shows fairly severe rickets in a rib There is considerable broadening of the zone of ossification which appears to have no definite margins The structure of both bone and cartilage is irregular

I think there can be no doubt about the changes This shows that a large proportion of a series of European infants show unmistakable histological signs of rickets even at birth while the majority develop them during the first weeks of life Such signs are not confined to premature infants but are equally common in full term babies dying at this age There is nothing to suggest that any of the mothers suffered from osteomalacia They came from all social classes and their diet was stated to be satisfactory and to include large amounts of milk Most of them however did not take any vitamin D preparation during pregnancy

The surprising finding of so much rickets in quite newborn infants now raises some questions which have to be answered It has to be explained first why this series contains so much more rickets than does the series of

Follis Jackson and Park Second it has to be investigated at how early a stage signs of rickets may be found when they can be found in 35 per cent of all newborns then it must be possible to make similar findings sometimes in older infants too And third it has to be asked if the changes observed really mean rickets or if they have any other explanation

My co workers and I are now trying to find an answer to these questions We are collecting a new series of ribs gathered from three different parts of Sweden from Sundsvall on the sixty third degree of latitude from Uppsala on the sixtieth and from Malmo on the fifty-sixth By comparing the collections



Fig 53 Normal costochondral junction (Ranstrom S and von Sydow G Rickets in New born Infants" *Pediatrics* 4 408 1949 Charles C Thomas Publisher)

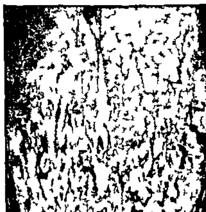


Fig 54 Costochondral junction in rickets (Ranstrom S and von Sydow G Rickets in Newborn Infants" *Pediatrics* 4 408 1949 Charles C Thomas Publisher)

from northern and southern places we hope to get an opinion on whether the amount of insolation or the living conditions of the mothers play any role in the development of the changes and thus might explain the difference between the findings in Baltimore and in Goteborg In this series we are including stillborn infants and abortions down to a weight of about 100 gm We are also using a different histological technique with only partial decalcification since some objections have been raised against our former technique with total decalcification

The collection of this series is still going on and I am not able to give any figures from it now The only result I can report today is that there are lots of pictures suggesting rickets even in fetal life Some more pictures from newborn premature infants prepared with our new technique by which the

osteoid is visualized illustrate this Figure 55 shows slight rachitic changes the line of ossification is straight The cartilage cells show a slight hyper plasia hypertrophy There are thin zones of osteoid covering the bone trabeculae Figure 56 shows broadened diffuse zones of ossification with

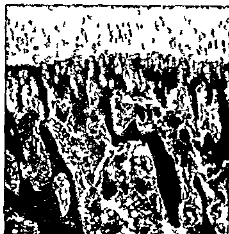


Fig 55 Osteoid tissue in mild rickets

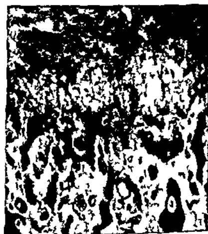


Fig 56 Zones of ossification in rickets



Fig 57 Bone trabeculae in rickets

strong hyperplasia hypertrophy of the cartilage cells irregularly structured bone trabeculae and osteoid Figure 57 is a higher magnification of a similar picture you can see the mighty margins of osteoid in the periphery of the bone trabeculae I think it cannot be denied that this is histologically rickets

It must be admitted however that we have not proved that the changes found in these two investigations really mean rickets but only that the histological picture is the same as is usually recognized as rickets We have not established whether the rachitic changes are connected with living conditions of the mothers or of the fetuses which may be supposed to cause such shortage in mineral or vitamin supply as is known to bring about rickets in infants We hope to find an answer to this question in our present investigations I may be

allowed however to draw a parallel to some results obtained during an investigation of some chemical and roentgenological conditions in premature infants which I am going to report in more detail at the coming panel on cow s milk versus breast milk

In Figure 58 the serum values of phosphatase of inorganic phosphorus and of calcium are illustrated in full term and premature newborn infants examined during the first three days of life The columns represent the means plus or minus the standard deviation a short column lying near the bottom of the diagram thus represents a low mean with a small variability The striped columns represent the values in normal full term infants the blank columns premature infants weighing 2000 gm or less at birth It is seen that already in these early days of life the serum calcium is lower in the premature infants the difference being about 1 mg per cent with more than twice as large a variability as in the normal series The serum phosphatase is markedly higher with a larger variability in the premature series while there is no definite difference in the phosphorus values

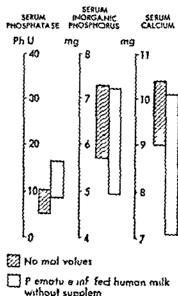


Fig 58 Serum phosphata e phosphorus and calc um in newborn full term and premature infants

In the following months the difference between full term and premature infants is highly dependent on the feeding of the premature infants In Figure 59 the black columns refer to normal full term breast fed infants given the usual supplements of vitamin D from birth The blank columns refer to premature infants fed exclusively on breast milk the stippled columns to premature infants receiving breast milk with a large supplement of vitamin D the striped columns to premature infants receiving breast milk with a supplement of cow s milk while the cross hatched columns refer to premature infants receiving both vitamin D and cow s milk It will be seen that in premature infants receiving exclusively breast milk the phosphatase concentration is very high and the phosphorus and calcium levels low and the variability great in comparison with the values of full term infants Supplementation with vitamin D does not affect the phosphatase and phosphorus

levels but raises the calcium level while supplementation with cows milk which means in effect supplementation with minerals brings the levels of phosphatase and phosphorus nearer to normal while leaving the calcium level unaffected Finally supplementation with both vitamin D and cows milk causes the values for all three substances to approach normal

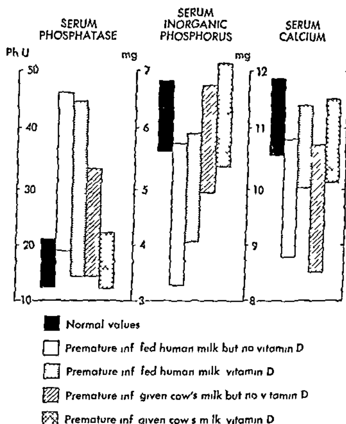


Fig 59 Effect of diet on serum phosphatase phosphorus and calcium in premature infants (von Sydow G *Acta paediat* 33 [suppl 2] 73 1946)

From this it may be seen that in the mineral conditions immediately after birth there is a certain difference between premature and full term infants and that this difference will be strongly increased during the first few weeks of life if the premature infants receive a diet short in minerals and in vitamin D The difference may on the other hand be compensated by giving the premature infants a supplement of minerals and vitamin D It seems reason

able to assume that the difference at birth may be caused by conditions similar to those increasing it after birth

In the light of the experiments of Benjamin and co workers and of Professor Nicolaysen I have felt justified in interpreting my results in premature infants after birth as meaning that the premature infant receiving breast milk is not being given enough phosphorus for the proper development of the skeleton The amount of calcium is sufficient but it is not absorbed from the gut unless vitamin D is given as well Supplementation with cow s milk raises the intake of phosphorus while the addition of vitamin D ensures the absorption of the calcium Of course the shortage in minerals of the breast fed will be especially pronounced if the mineral reserves at birth are poor as is usually the case in premature infants

Now if the changes we have found in fetuses should be proved to be real rickets then this theory must be widened to comprise also processes taking place before birth It seems to me that a theory of the pathogenesis of early rickets then might be formulated as follows

The mother normally lays up increased reserves of minerals in her own body and in the fetus during pregnancy Some of these are used in the development of the fetal skeleton and if enough vitamin D is present normal bone is formed The greater part of the calcification of the fetal skeleton occurs during the last months of intrauterine life Therefore if a child is born prematurely its mineral reserves are often insufficient The same is true if for some reason or other the mother cannot store minerals properly in her own body or in that of the fetus Even so if enough vitamin D has been available during intrauterine life the child may not be born with rickets but it is very likely to become rachitic shortly after birth especially if it is still receiving insufficient minerals as is the case in breast feeding If on the other hand there is a deficiency of vitamin D during fetal life the child will be born with rickets In either case therapeutic doses of vitamin D are required to cure the rickets or check its progress

If this is true—I repeat that there are still some gaps in the deduction—then preventive measures should be directed toward the very earliest stages of development far more than has hitherto been the practice We have all seen children who develop rickets in spite of the ordinary antirachitic measures Perhaps this is because the rachitic process had started in them before birth and they ought to have received therapeutic doses of vitamin D and minerals to heal them instead of the prophylactic doses required by other children to prevent them I think the following are then the lines along which we should try to prevent rickets

Pregnant women should receive extra vitamin D and minerals Infants of mothers who have not received this treatment should receive therapeutic doses of vitamin D from birth Premature infants and others where there is a suspicion that the mineral reserves are low should if breast fed receive extra minerals The full term infants of mothers who have received antirachitic treatment probably need much less vitamin D during early life than do those whose mothers have not had such treatment They also probably need less minerals than premature infants Further investigations will decide whether this view is right and if so how large are the amounts needed

Professor LEVINE (New York) I think this is a really beautiful piece of experimental work Except for the quantitative aspect I think Dr von Sydow s approach has solved a good deal of the problem Is it fair to assume that the calcium in the serum is a true representation of the calcium stored in the bone?

Professor MELLANDER (Goteborg) I would like to ask what is the normal level of phosphatase in premature infants?

Dr von SYDOW (Sundsvall) In a series of normal breast fed infants I found a mean of 7 5 units (method of King Hansen) for the first week of life after the second to about the fourteenth week of life the period which I have investigated 6 5 units

Professor MELLANDER (Goteborg) But that refers to full term infants When you consider the phosphatase content in prematures to be higher than normal it would be interesting to know how high is the normal level for a *premature* infant

Dr von SYDOW (Sundsvall) I cannot answer that in any other way than by recalling my figure in which it was shown that the phosphatase level was highly dependent on the type of feeding in the premature infant It was very high in those receiving exclusively breast milk and it was also high in those receiving breast milk plus vitamin D It was much lower in those receiving a supply of cow s milk and it was normal in those receiving both cow s milk and vitamin D I think it will be normal in premature infants given sufficient amounts of minerals and vitamin D

Professor MELLANDER (Goteborg) What is most normal a premature infant fed human milk or a premature infant fed cow s milk?

Professor RAIHA (Helsinki) It is known that there is a difference in level during fetal life in different enzymes Is it then certain that the normal values of phosphatase also are the normal values for prematures? We have looked at cocarboxylase and find that in a pregnant woman this is about 8 μgm per cent and in cord blood about 14 μgm per cent So if there is a difference in one enzyme perhaps there is also a difference in another enzyme

Professor WALLGREN (Stockholm) I am afraid we are going too much into the discussion on premature babies We have two separate days to discuss that matter Shall we now adjourn this panel until tomorrow morning?

Professor NICOLAYSEN (Oslo) Does anybody want to say anything about the discussion yesterday?

Professor BESSEY (Chicago) There is a point in your introductory talk of yesterday morning concerning adaptation to low calcium intake that I would like to speak about Some years ago I did an experiment in which groups of rats were placed on varying levels of calcium intake throughout their lifetime At the highest levels of intake the animals brought their body calcium to the maximum adult percentage of body weight As the levels were decreased they still reached this final percentage but a little later in adulthood Finally a level was reached at which the first generation of rats was unable to reach a normal percentage of calcium in their skeleton Thus after a certain stage of maturity they seemed to have lost the chance of bringing the calcium up to the adult level

If the second generation of this latter group of animals were kept on this low level of calcium intake they managed to reach a normal body percentage of calcium But they did this by an adaptation in which the skeleton is smaller than normal They look like perfectly normal creatures but their bodies are short although the percentage of body calcium is normal I would interpret this as an example of how if one continues to feed calcium at a certain low level so that you do not completely interfere with development one can produce animals which adapt themselves by the second generation to a lower calcium intake at the expense of size of skeleton

Professor NICOLAYSEN (Oslo) It has been told me that Chinese people who have been transferred to the United States have within a generation or two increased about 6 or 7 in in height Obviously you cannot discuss that only as a calcium problem but it is well known that the Chinese diet is exceedingly low in calcium Has anyone any idea if there is any link between calcium or any other specific nutrient in the Chinese diet as contrasted with the American and this striking increase in growth when the Chinese are transferred to an entirely different diet?

Professor BESSEY (Chicago) I believe that this point you raised is in connection with Japanese not Chinese The statistics are those that come from measurements of students in California compared with brothers sisters or cousins who remained in Japan These comparisons show that the

Japanese who came to California were within a generation distinctly taller individuals than those who remained in Japan. These are not the most exact kind of figures and they may be subject to some uncertainty but I think that they are probably significant. If one compares the diet of Japanese in Japan with that of the Japanese in California two factors seem different. The diets in Japan tend to be short in calcium and in proteins. In California that situation is very much improved.

In this connection it might be of interest that in studies that were done on children during the Spanish Revolution the most striking observation was that the children had markedly shorter skeletons although they looked healthy. This amounted to 3 or 4 in over a period of three years. Calcium and proteins were low in their diets. Another interesting point was that the hemoglobin levels in these children were very high, as much as 15 gm per 100 ml. I do not know what the explanation is for this latter fact. Ascorbic acid levels incidentally were excellent. They had been living for the most part on green beans, a good source of iron and ascorbic acid but low in calcium and apparently low in protein.

Professor WALLGREN (Stockholm): We had the same experience about decreased rapidity of growth in Sweden during the First World War. Stockholm children were found to be distinctly less tall on the average than before the war. After the war they increased more rapidly than usual and after about two years time they had attained the normal height that other children had before the war at the same age.

Professor NICOLAYSEN (Oslo): We might say that the problem is how is chemical growth linked up with morphologic growth and I think that we should encourage the World Health Organization to have a separate seminar on the growth problem.

Professor WALLGREN (Stockholm): We talked about the requirements of vitamin D and yet there are vitamin D–resistant cases of rickets. How are these cases to be explained?

Professor STEARNS (Iowa City): I think the very existence of resistant rickets can be taken as proof that something more than the effect of vitamin D on the gastrointestinal absorption of calcium is required because these children can absorb calcium and phosphorus well. They will get healing of the rickets without any increase from the original low level of inorganic phosphate but with a striking increase in the level of water-soluble organic phosphates. Therefore it seems to be that the vitamin D must have some effect within the body, as other evidence also indicates, beside its effect on absorption in the gastrointestinal tract.

Professor WALLGREN (Stockholm): Has the liver an important role

in the metabolism of vitamin D perhaps of the same sort as the liver has with respect to vitamin K?

Professor STEARNS (Iowa City) Hepatic rickets has been shown in various places but we have not found such a child with what we call true hepatic rickets We have seen two adolescent children and several women with osteoporosis of menopause in whom we were unable to get a satisfactory absorption and retention of calcium even with a diet very low in fat and with what we considered an ample amount of vitamin D After the administration of bile salts however these people were able to absorb adequate amounts of calcium and to retain it for the manufacture of bone

We have used that type of therapy fairly frequently particularly in our clinic for orthopedics where they have many women with osteoporosis and it does seem to be useful Whether it increases the absorption of vitamin D or whether it increases the absorption of the calcium or phosphorus I do not know

Dr ZETTERSTRÖM (Stockholm) Samuels and collaborators have been able to show the existence of an enzyme system in the liver which is capable of destroying testosterone by oxidation Possibly such a process occurs in the liver and destroys vitamin D in the cases of D resistant rickets

Professor LEVINE (New York) In Belgium France and occasionally in the United States vitamin D intoxication in infants has been reported What is the situation in the Scandinavian countries?

Professor WALLGREN (Stockholm) We have seen some cases of hypervitaminosis D due to an obviously excessive supply of vitamin D The children suffered from renal insufficiency but eventually recovered A thing that puzzled me a little in the talk yesterday by Professor Nicolaysen was his statement that in hypervitaminosis there was a depletion of calcium in the bones We see however in the X ray photographs an increased calcification of the epiphysis and of course there is an increased amount of calcium in the blood

Professor RÄIHÄ (Helsinki) There is one case known in Finland The child died and at autopsy calcification of the kidney was found

Professor WALLGREN (Stockholm) There is that peculiar difference in susceptibility to intoxication from the vitamin For instance in a case of vitamin D-resistant rickets in a child of one year we had to give doses of 500 000 units of vitamin D daily for 3 or 4 weeks and there were no signs of intoxication Certainly if you had given that to another such small child he would have developed symptoms of intoxication It shows too that there is perhaps something in the idea of Dr Zetterstrom that vitamin D is destroyed in the liver or elsewhere in the body

Dr CLEMENTS (Geneva) We had a very good discussion at Leyden on the practices in the various countries represented at the seminar Would representatives from the various countries here indicate what is common practice in their country in the use of vitamin D in the first year of life or throughout the growing period of life

Professor WALLGREN (Stockholm) It is of course a very difficult question to answer generally because there are very many other factors that interfere with the chosen dose of vitamin D given generally to infants and children such as amount of sunshine and daylight and I do not think that in our country there is any general practice about this I suppose that in the far north in mid wintertime they give higher doses of vitamin D Here in Stockholm physicians generally give about 1000 units of vitamin D preparation from halibut liver oil daily starting from the first week of life from October until May They give nothing generally to full term infants during summertime To premature infants we give generally and in my clinic as a routine a massive dose of 500 000 units of vitamin D and we expect that this will last 2 to 3 months Then we start with this dose of 1000 units daily and we give children up to about 3 years of age about the same dose of vitamin D daily From 4 to 7 and in school it is not given as a routine but we usually give it to these groups temporarily during the wintertime and also to school children

Dr CLEMENTS (Geneva) It is not the practice I take it then from what Professor Wallgren says to give doses of 15 mg (300 000 international units) by mouth several times a year to children as it is in some countries in Europe

Professor WALLGREN (Stockholm) No

Professor RAIHA (Helsinki) In Finnish Lapland the natives use cod liver oil in cooking When they fry anything they always use cod liver oil

In Finland we use between 1000 and 2000 units of natural cod liver oil daily beginning at the first two months of age

Professor SIWE (Lund) At the last meeting of the Society of Pediatricians we discussed this problem Our medical board recommends a dose of 500 units a day for infants and many of us have had the experience that such a dose is not sufficient for all of them A rather large percentage will show rickets even if they get that dose So we agreed to double the dose and give 1000 units as we have done for a very long time in the south of Sweden

Mention was made of the effect of sunlight In all our districts during the winter there is none at all By direct measurement you can see that the short waves will not come down to the earth s surface We have learned this result by experience in our clinical investigations on children too We must

give all children I think a prophylactic dose and that must not be lower than 1000 units daily during the winter season There will be some cases in which even that dose will not suffice We have had several cases in which we must give two or three times as much but they are rare In this connection I have in mind the fact that the calcium and phosphorus intake level of the child plays a very definite role in the amount of vitamin D necessary to keep rickets away

Dr von SYDOW (Sundsvall) I live in the north of Sweden and have come to the same conclusion as Professor Wallgren that there they need more vitamin D than in the south of Sweden I agree with Professor Siwe that in winter there is no effect of ultraviolet rays of the sun in some parts of Sweden The winter is longer in the north than in the south and therefore we have to give vitamin D for a longer time beginning with the first week of life and from September or October to June In a few cases where it is obvious that we cannot trust mothers to give their children a daily dose of vitamin D we give the child a massive simple dose of 500 000 units

Dr CLEMENTS (Geneva) Professor Siwe said that all children must be given prophylactic doses of vitamin D I wonder if he could expand that and say whether this is done up to the end of the growing period or whether by children he means infants In some cases 1000 international units daily were found to be insufficient and it was necessary to give twice or three times that dose Is the criterion the development of rickets?

Professor SIWE (Lund) It was because they developed rickets in spite of having gotten 1000 units daily Then we gave of course treatment doses of 15 000 to 30 000 units daily I meant that even with 1000 units daily we could not be sure that all children would not get rickets

Dr CLEMENTS (Geneva) When you use the term all children what does that mean? Does it mean that you give vitamin D up to the end of the growing period or during the first two years of life or to what age?

Professor SIWE (Lund) I give it to all children during the first five years During school age I do not find it necessary to give it to all of them but I do give it to a rather large number of the school children too if they show any signs of not attaining proper height or poor appetite or a slowing down of physical or psychic development

Dr CLEMENTS (Geneva) Is that a systematic distribution to all homes?

Professor SIWE (Lund) Yes

Professor YLPPO (Helsinki) To our premature children we usually give in the first 2 to 3 weeks intramuscular injections of 500 000 units of D and repeat these injections every 2 to 3 months Using the small dose of 6000

to 12 000 units which Gerstenberger used we could not prevent rickets in Finland

METABOLISM AND MOLECULAR STRUCTURE OF MINERAL SALTS IN BONE TISSUE DURING GROWTH AND CERTAIN PATHOLOGICAL CONDITIONS

Dr ENGSTRÖM (Stockholm) *Introduction* This paper is a summary of some experimental approaches to the problem of correlating the

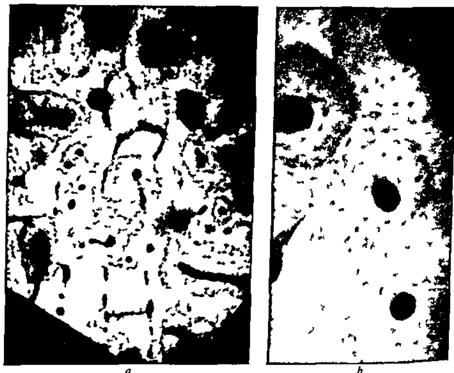


Fig 60 Enlargements of microradiograms of bone tissue Cross sections about 30 μ thick from the compacta of a dog (*a*) and horse (*b*) The osteocytes are less calcified than the rest of the bone tissue Haversian systems of different degrees of calcification are seen X rays of 6000 volts filtered in 1 mm beryllium were used The white areas are more X ray absorbing than the black Magnification (*a*) C 80× (*b*) C 200×

morphology with the physiology of bone tissue The techniques used were the following (1) determination of the distribution of mineral salts in thin ground sections of bone tissue by microradiography (2) metabolic studies of different structures in bone tissue using P³ and autoradiography (3)

analysis of structure and orientation of the mineral salts in bone using an X ray diffraction microtechnique Full accounts of the methods are given in the papers of Amprino and Engstrom (1950) Engstrom and Amprino (1950) and of Engstrom and Engfeldt (1950)

Results The lacunae of the osteocytes can be seen in the X ray microradiograms Figure 60 as oval or spindle shaped areas transparent to X rays The matrix has the strongest X ray absorption the collagen less A relation

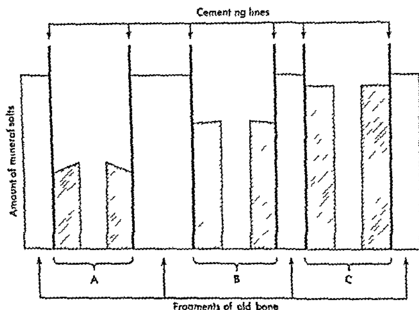


Fig 61 Schematic drawing showing steps in the formation of a Haversian system The high content of mineral salts close to the lumen of an osteon indicates a central deposition of minerals (Modified from Amprino and Engstrom)

seems to exist between the average absorption of ground substance and the ratio of the amounts of collagen to cementing substance The more abundant the latter is the more absorption there is in the whole matrix When the sections were decalcified no contrast at all was obtained in the microradiogram indicating that in ordinary ground sections the microradiogram shows the distribution of mineral salts

When examining a microradiogram of bone the most striking feature is that the distribution of mineral salts is extremely uneven The secondary bone such as Haversian systems (osteons) has less absorption than primary bone Furthermore there is a correlation between the amount of absorbing

substance and the age of the osteon The younger the osteon the smaller its content of mineral salts The distribution of calcium salts is not uniform, either within each single Haversian system or in a fragment of the latter The X ray absorption is invariably higher in the inner zone which borders the lumen of the channel and decreases gradually towards the periphery of the system The cementing line which is easily seen in bones from certain animal species has a very high absorption of X rays In the X ray pictures

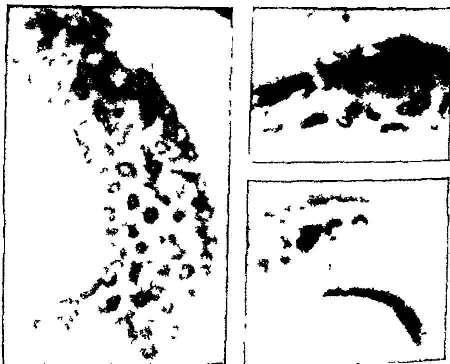


Fig 62 Autoradiographs of C 39 μ thick sections of compacta of different bones in a dog which had received 10 microcuries of P^{32} three days before it was sacrificed The black areas indicate high concentration of P^{32}

the borders of the resorption cavities are quite sharp The marginal layer lining the cavity even where actively resorbing osteoclasts are present absorbs as much as does the neighbouring tissue The results are summarized in Figure 61 which shows steps in the formation of a Haversian system A differential count of Haversian systems with different content of calcium salts indicates that in the first period of the formation of a system the deposition of calcium salts seems to be faster than in the later stage

In order to check the findings mentioned experiments with the localization

of P ² were performed Two dogs were injected with a high dose of radio active phosphate One dog was killed after 3 hours and the other after 4 days Ground sections 30 to 40 μ thick were prepared and laid into good contact with a fine grained photographic film In both animals the radioactive phosphorus was concentrated in certain Haversian systems

By taking an X ray microradiograph of the same section that had been autoradiographed it could be shown that those Haversian systems which had the smallest content of mineral salts had the highest uptake of P^{32} Autoradiographs are shown in Figure 62 The analysis of the distribution of P ² together with the distribution of mineral salts is given in the diagram in Figure 63

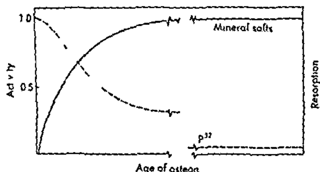


Fig 63 Schematic picture showing the changes in content of mineral salts and uptake of P^{32} in osteons of different age (Modified from Amprino and Engstrom)

As can be seen from Figure 63 the first process of the formation of an Haversian system is relatively rapid The rate of deposition of mineral salts then slows down at the same time as the concentration P^{32} diminishes

The ultrastructure of the bones was examined by the study of the X ray scattering (Table 3) In Figure 64 copies of the X ray diffractograms are shown The microtechnique described by Chesley was used and the area subjected to diffraction analysis was selected microscopically and could also be localized in the microradiograms The diffraction pattern from normal bones bones which had grown under immobilization (Engstrom and Amprino 1950) and bones from animals treated with great doses of parathyroid hormon (PTH) all showed the same diffraction pattern and the same orientation (fibering) in longitudinal sections No orientation was found in cross sections

Conclusions During analysis of the absorption and diffraction of X rays

TABLE 3
Interplanar Spacings of Bones (A)

Dog			Man			
Normal Radius	Immobil Radius	PTH Radius	Young Osteon	Old Osteon	Bone Tumor	New born
3 76	3 76	3 53	3 70	3 77	3 74	3 66
3 39	3 35	3 26	3 34	3 38	3 38	3 37
3 08	3 09	2 98	2 98	2 99	3 07	3 07
2 74	2 72	2 68	2 71	2 74	2 74	2 73
2 61	2 58	2 54	2 60	2 62	2 67	2 57
2 25	2 22	2 25	2 22	2 24	2 24	2 23
2 05	2 03	2 02	2 02	2 04	*	2 00
1 93	1 91	1 91	1 91	1 92	1 91	1 89
1 82	1 82	1 83	1 80	1 82	1 79	1 79
1 70	1 70	1 69	1 69	1 70	*	1 68

* Too weak to be measured with accuracy
PTH = treated with parathyroid hormone

in bone material the changes in mineral composition and in ultrastructure could be correlated with the continuous reconstruction of microscopic bone structure

The bundles of collagen fibers are less calcified than the surrounding amorphous organic substance of bone matrix The distribution of calcium in the bone tissue is not uniform The secondary bone formed through re construction has less content of mineral salts than primary bone The calci fication of secondary bone increases gradually but the speed seems to be

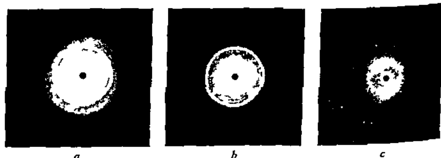


Fig 64 X ray diffractograms of bone tissue (*a*) longitudinal section through a Haversian system (note the orientation in several rings) (*b*) cross section of a Haversian system no orientation (*c*) decalcified longitudinal section showing traces of one mineral ring and the organic reflections

higher in the beginning The increment of calcification of secondary bone is more rapid in the parts of matrix which lie close to the vascular channels

In secondary bone Haversian systems the uptake of radioactive phosphorus is higher in those systems which have a low content of mineral salts than in those with a high content of mineral salts

The ultrastructure of bones under different growth conditions during immobilization and when subjected to decalcification by parathyroid hormone as judged by X ray diffraction seems to be the same

The results of the present investigation show that the substitution of primary with secondary bone is *not* a process by which the physical resistance of bones to mechanical stresses necessarily increases The rebuilding of the structures of second order must rather be viewed as a process which allows a continuous fixation and release of organic and inorganic substances

Professor NICOLAYSEN (Oslo) When one considers bone and its breaking strength perhaps there is an inclination to feel that the breaking strength is due to lines of force between crystals But there must be very strong lines of force between the crystals and the protein molecules I judge this from the effect of high doses of vitamin A on the bones where you get spontaneous fractures without reduced ash content and apparently without diminution of the diameter of the cortex I think the same picture is seen in scurvy In both cases apparently you have an effect on the structure of the protein molecule with an essential change in the binding forces between the crystals and the proteins

Professor MELLANDER (Goteborg) When we discuss phosphorus and calcium metabolism of infants it may be of value to consider not only that milk is the only nutritional source for these minerals during the first period of life but also that the most characteristic component of milk is a phosphoprotein the casein Where phosphoproteins occur in nature they are always connected with nutrition of very young individuals or fetuses About 25 per cent of the total amount of phosphorus in cow s as well as human milk is casein phosphorus We have thought it worth while to see if this phosphorus is of any special importance in the mineral nutrition of the suckling and especially if the absorption of this phosphorus is in some way or other connected with the absorption of the milk calcium First of all it is clear that when casein is hydrolyzed by the proteolytic enzymes of the digestive tract most of its phosphorus (for cow s casein at least 50 per cent and for human casein 70 per cent) is turned over to certain peptides of very constant amino acid composition (glutamic acid serine asparagine threonine valine and small amounts of glycine alanine leucine isoleucine) The number of amino acid residues of these peptides seems to vary a great deal

but they have one thing in common they are resistant toward proteolytic enzymes as long as they are phosphorylated This together with the fact that they form very soluble calcium salts with a calcium content of as much as 10 per cent suggested that they might possibly be a physiologic carrier of calcium as well as phosphorus in the milk fed baby In order to test this hypothesis we have so far investigated to what extent these compounds can be absorbed from the intestinal tract under different conditions and also what happens after intravenous injection of calcium and phosphorus in this form

The first experiment shown in Table 4 concerns a boy aged 6 months

TABLE 4

Time of experiment 6 days The balance experiment was begun 2 weeks after admission

Dosage 30 gm Ca phosphopeptide = 3 25 gm Ca
Via the food circa 2 9 gm Ca

Secreted in feces 1 76 gm Ca
Secreted in urine 0 005 mg per ml Discounted
Absorbed from peptide Ca
(a) if all Ca from the food is assumed absorbed 1 49 gm = 46 per cent
(b) if 50 per cent Ca from the food is assumed absorbed 2 49 gm = 90 per cent

	Before experiment	After experiment
Symptoms of rickets		
Craniotabes	++	+
Epiphyseal swelling	+	+
X ray	Flourishing rickets	After 7 days distinct calcification
Alkaline serum phosphatase (Bodansky units per 100 ml)	50	20
Serum Ca (mg per cent)	9 2	9 2
Electrical threshold (in milliamperes) C O C	Over 5	—

with clinically diagnosed rickets This boy was breast fed only for the first month received mixed feedings during the second month and from the third month nothing but cow s milk He received no vitamin prophylaxis except for three days immediately before admission to the hospital The feeding during the balance period and for two weeks before it was an 800-gm half milk formula daily Even allowing for the most unfavorable possibility i e that all the dietary calcium was absorbed and consequently all the fecal calcium must have come from the calcium phosphopeptide there is still an absorption of 46 per cent of the peptide bound calcium If 50 per cent of the dietary calcium is assumed absorbed the retention can be calculated to 90 per cent The actual absorption thus must be within these extremes

chickens is superior to the antirachitic action of the same amount of calcium and phosphorus in inorganic form

Professor NICOLAYSEN (Oslo) Your peptide contains about 9 per cent calcium and 6 per cent phosphorus What I should like is the interpretation I did some tests for you with the isolated loop technique and found in comparison experiments with other calcium sources no difference in the absorption of calcium But am I not right in saying that what I found was a remarkably high absorption of phosphorus? Nearly all the phosphorus could be split off and absorbed from this compound in isolated loops and the

TABLE 6

Evaluation of the Antirachitic Effect of Calcium Phosphopeptides from Cow s Casein on Chicks According to the Method of N Olsson

For all Groups Rachitogenic Standard Diet and Suboptimal Addition of Vitamin D

Tmt Values Higher Than 1 Indicate Rickets

Group	Number of animals	Ca and P added as	Tmt values
I	8 + 7	bone meal	3 16
II	6 + 9	bone meal Ca Ppept	2 61
III	8 + 7	bone meal Ca Ppept	2 73
I	9 + 9	bone meal	2 99
II	9 + 9	bone meal Ca Ppept	2 53
III	8 + 10	bone meal Ca Ppept	2 10

explanation of the effect might be that you have to get the calcium and the phosphorus in a form that can keep calcium and phosphorus in solution Did you use a high-calcium diet in your rats? Then I would suspect that the effect in the rats was an effect of an increased absorption of phosphorus due simply to the fact that calcium was prevented from getting into ionic contact with the phosphorus

Professor MELLANDER (Goteborg) Perhaps it should have been pointed out that this calcium salt of the phosphopeptide is extremely sensitive to alkaline phosphatase If in the isolated loop there is any phosphatase activity the result will be that the phosphorus splits off and the calcium is precipitated and cannot be absorbed

have an increasing percentage retention with increasing intake This seems rath r surprising because customarily we think that the young infant is less efficient than the older infant yet here we find that young infants with their higher per kilogram intake of protein use it more efficiently than older infants The amount of intake is high going up to a maximum of 4 to 5 gm of protein

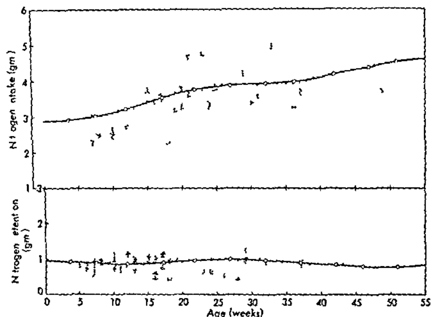


Fig 6. Intake and retention of nitrogen by infants fed cow s milk Data in grams per 4 hours

per kilogram and yet it is used very efficiently This means that a young baby has a capacity to digest and absorb many times the amount of protein that he would normally be fed in human milk and that this capacity apparently is at its greatest in early infancy

Figure 69 shows the utilization of nitrogen by infants with a mild gastrointestinal disturbance that is not a severe diarrhea but an increase in the number of stools or a mild infection such as a cold Such mild infections in these infants resulted in very poor retention of calcium or even a loss of calcium from the body But as is shown they made almost no difference in the absorption and retention of nitrogen which was still very good These data confirm earlier studies of Utheim in infants with marasmus who she found were able to use protein with extreme efficiency

Figure 70 shows the nitrogen intake in milligrams per kilogram and the

CHAPTER III

Panel on Protein Metabolism

NITROGEN METABOLISM IN INFANCY

Professor STEARNS (Iowa City) Our studies on the nitrogen metabolism of infants were made on the same groups of babies studied for calcium and phosphorus retention and again cover the major part of the period of infancy for each baby These babies were fed largely cow s milk usually undiluted with a 6 per cent carbohydrate addition and the milk suitably curded by boiling or by addition of acid in various ways We have very few studies of human milk and the one figure of babies fed human milk contains data gathered very largely from the literature

Figure 65 shows the daily retention and mean curves for daily intake and retention of nitrogen by infants listed according to age in weeks The nitrogen intake increases from about 3 gm in the first five weeks up to 4 5 gm toward the end of infancy but the total daily mean nitrogen retention remains surprisingly constant almost to a gram tending to dip a little towards the end of the first year That means of course that the young infants are more efficient in their utilization of the nitrogen and retain a higher percentage of the nitrogen fed than do the older infants

Figure 66 shows the same data in a little different order The first curve at the top is the daily intake The second curve is the retention in percentage of intake starting at a little over 30 per cent of the intake for the very young infants and dropping to about 10 per cent of the intake toward the end of the first year The lowest curve is the increment of gain in weight which also tends to slow very markedly Figure 67 shows the same data calculated in milligrams per kilogram intake on the abscissa and retention on the ordinate and shows a directly proportional relationship between intake and retention As a rule of course the youngest infants have the highest intake per kilogram although there is some spread of the data

Figure 68 shows that even when calculated on a per kilogram basis we still

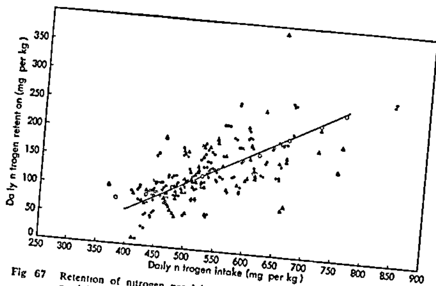


Fig 67 Retention of nitrogen per kilogram of body weight in relation to intake per kilogram Infants from 3 to 50 weeks of age

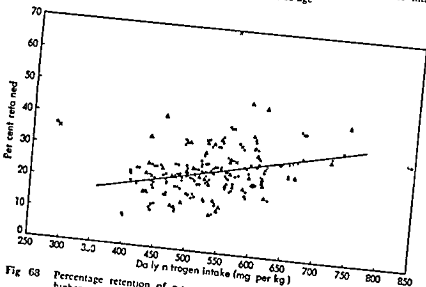


Fig 68 Percentage retention of nitrogen calculated on per kilogram basis shows higher percentage of retention from higher intakes The highest intakes per kilogram tended to be among the younger infants

retention in milligrams per kilogram of babies fed human milk Here again we see that there is a relation between intake and retention and that the percentage retention for intake increases with intake Babies reach 150 mg nitrogen retention before they are taking quite 400 mg of nitrogen per kilo-

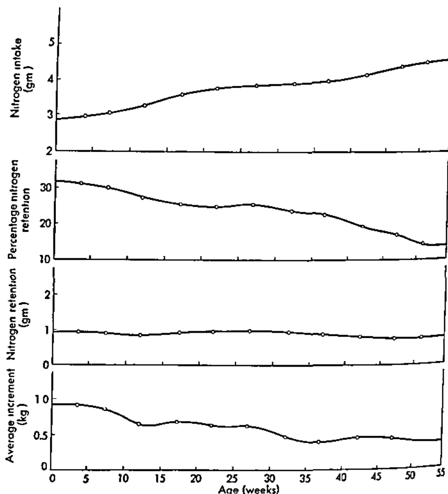


Fig 66 Infants fed cow s milk their average daily nitrogen intake retention percentage of retention and the weight increment in relation to the age of the infant

gram (38 per cent retention) and have an average retention of only about 65 mg with 200 mg (32 per cent retention) Thus babies fed human milk show exactly the same phenomenon as those fed cow s milk but the percentage retention is always much higher as one might expect

mean daily nitrogen retentions during that period to the nitrogen percentage at birth we can calculate the nitrogen percentage of each infant at the given age We find that the baby fed human milk tends to maintain his birth content of nitrogen and the baby fed a higher protein intake continues to increase his birth content of nitrogen at a rate practically comparable with the rate of growth The calcium curve (Fig 49 Chap II) soon after birth shows a period of adjustment A newborn baby is not able to maintain his birth content of calcium for a period of about 6 to 8 weeks regardless of the amount

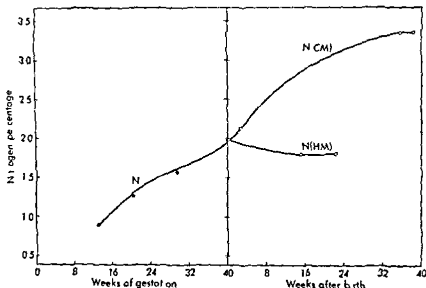


Fig 71 Nitrogen content of the infant before and after birth *HM* infants fed human milk *CM* infants fed undiluted cows milk suitably curded (Modified from Stearns, G *Phys ol Re* 19 4 1939)

fed or of whether vitamin D is given or not Apparently there is no such difficulty for the newborn infant in retaining nitrogen The nitrogen retention is ample almost immediately It leaves one wondering why the young infant has a capacity to utilize protein in amounts far beyond that which he could possibly obtain from his natural food

Figure 72 is a graph of mean retention against intake of nitrogen The top curve shows the mean retention during the first year of life in relation to the intake the second curve the mean retention in children from 1 to 2 years of age and the lowest curve the mean retention in children from 2 to 3 years of age These three groups show a decreasing ability to retain nitrogen from a

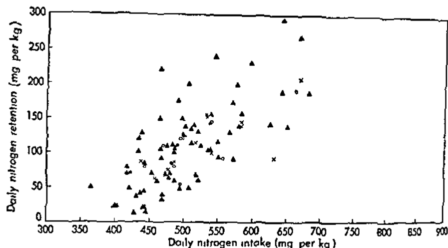


Fig 69 Relation of the daily nitrogen retention to intake in sick infants fed cow's milk

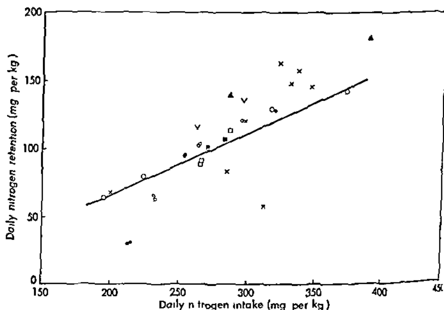


Fig 70 Retention of nitrogen by full term infants fed human milk.

In Figure 71 we attempted to find out what happens to this retained nitrogen The nitrogen curve during the weeks of gestation is taken from analyses of fetuses reported in the literature and the two curves for the weeks after birth are calculated by the method of Lindberg By measuring the time which each baby takes to gain 1 kg of weight and by adding the sum of his

solution containing only one protein is placed in a tube and an electric current is passed through this layer will migrate and its migration can be followed by observing the boundary If the solution is a mixture of say two proteins two boundaries will result each corresponding to the migration of one of the two different proteins Such a phenomenon can be observed by special optical arrangements

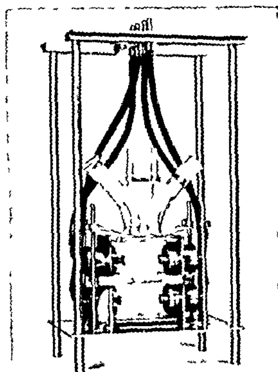


Fig 73 Apparatus for electrophoresis

Figure 73 shows the apparatus commonly used a glass U tube built up of sections specially made for this purpose The protein is layered in the lower half of the U tube from where its migration can be observed by suitable optical methods and registered photographically Figure 73 shows the essential part of the apparatus the electrophoresis tube itself without the optical and photographic equipment

Figure 74 gives a number of diagrams obtained from Edwin Cohn s laboratory in Boston on different protein fractions obtained by fractionating

given intake but at none of these ages do we have any evidence that we have reached the maximum retention of which the child is capable

In contrast the curve for children from 3 to 4 years of age shows an apparent maximum retention between an intake of 3 and 3 5 gm of protein per kilogram This same curve holds for children from 3 to 11 years of age

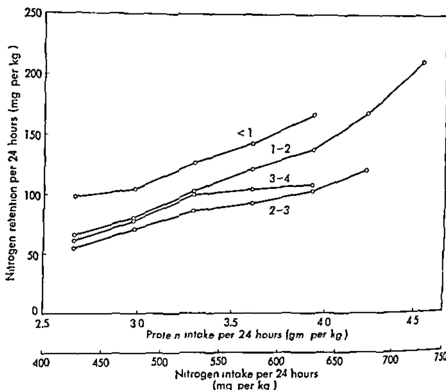


Fig 72 The mean retention of nitrogen of infants under 1 year of age and of children 1 to 2 years 2 to 3 years and 3 to 4 years of age plotted in relation to nitrogen intake Only after 3 years of age is a maximum retention observable at the intake levels attained

So from 3 years of age on the average child shows a tendency to a maximum retention But until then going up to beyond 4 gm per kilogram intake of protein we have no evidence that we have reached the maximum capacity of the child to absorb and retain proteins

SOME RECENT ADVANCES IN ELECTROPHORETIC TECHNIQUE

Professor TISELIUS (Uppsala) Electrophoresis is the migration of molecules or particles in an electric field in solution If a layer of a protein

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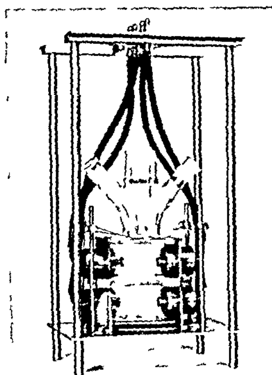


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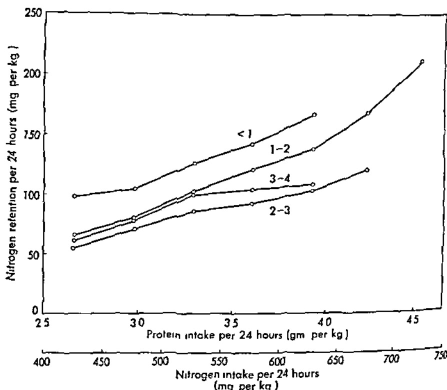


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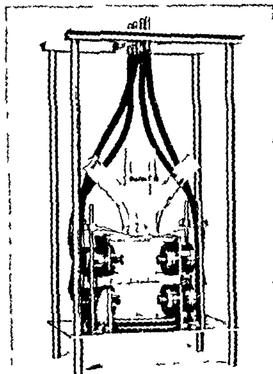


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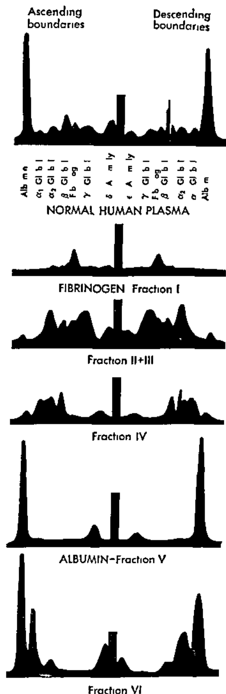


Fig 74 Electrophoretic patterns of normal human plasma and several plasma fractions

plasma by means of alcohol at low tem peratures There are a number of peaks in the upper diagram of unfrac tionated plasma The left and right halves correspond to the left and right hand limbs in the U tube Each of those peaks indicates the presence of one well defined protein or at least well-defined from the electrophoretic point of view In the lower diagrams one can see only one or two peaks cor responding to the fractions obtained This figure shows how such a method can be used as a guide in fractionation work very much in the same way as an inorganic chemist can use the spec troscope to follow fractionation of salts or metals

One may call this sort of electro-phoresis technique boundary electro-phoresis because one studies the boundaries It should be observed in this case that the separation although it can be observed very accurately is not a complete separation Rather it is a differential separation because all of the components except the fastest and the slowest overlap

Suppose a thin layer of the protein mixture is placed in the solution with buffer on both sides If a current is passed through and this layer contains two or three proteins two or three zones would be obtained This would be complete separation with the buf fer solution between the two or three zones and might be called zone elec trophoresis This method cannot be realized in solution in free electro-phoresis because each layer will nat urally be heavier than the surroundin

buffer solution since the layer contains protein These layers of heavier solution will gradually fall down and the whole contents of the tube will be stirred up

In the first electrophoresis experiments on ions and molecules in solutions by Hittorf in the 1860 s use was made of arrangements for immobilizing the solution in the tube in order to prevent the convection due to the layering of heavier material By introducing some layers of cloth he reduced this streaming and to a certain extent prevented the stirring caused by convection Many attempts have been made since to apply immobilizing material such as a filling in the tube to make this sort of separation possible One cannot do that without sacrifice but sometimes what is gained is worth this sacrifice Complete separation is gained and the possibility of optical observation is sacrificed The latter can be done in the first type of apparatus because this is a clear solution to look through One also sacrifices to a certain extent the possibility of measuring the mobility of the different proteins accurately and this is a property of the proteins essential to their comparison with one another Mobilities may be difficult to measure with a filling material because there may be all sorts of reactions between the proteins and the filling material With cloth paper or glass powder you know that proteins sometimes tend to become adsorbed very strongly However it is important if possible to measure mobilities in this case too so that the properties of some of the filling materials must be studied to determine whether there is a material which will not interfere very much with the migration

Such experiments have been made in many different laboratories and it is impossible here to go into details about all of this work I shall limit myself to a few improvements made recently in my own laboratory One way of trying this is to use a fairly coarse glass powder as a filling material because with a coarse powder adsorption is not so marked We have been able to confirm that in several cases The resulting apparatus looks very much the same as the usual boundary electrophoresis apparatus

In the center of Figure 75 is a U tube and to the left and the right you see the tubes for introducing the electrodes The left hand limb of the U tube is filled with glass powder with the zone of the protein solution introduced This is done before starting the experiment by applying slight suction to the tube One can let the tube dip down into the protein solution take up a small sample of the protein mixture and then let buffer solution follow After this the glass powder tube is placed in the apparatus as shown in the figure a current is sent through it and the zone migrates and eventually splits up into several zones if there is a mixture of several proteins The experiment is run for say eight hours Then the tube is removed and just as is done in chromatography buffer solution is allowed to enter at the top of the tube

slowly and the zones are washed out and collected in a sort of fraction collector Thus in a number of test tubes say 10 to 20 sections of the contents of the glass powder column are obtained and in them by suitable colorimetric methods or nitrogen analysis the components are found separated completely

This apparatus has been in use for several months and so far we have simply tested to what extent this arrangement interferes with the mobility

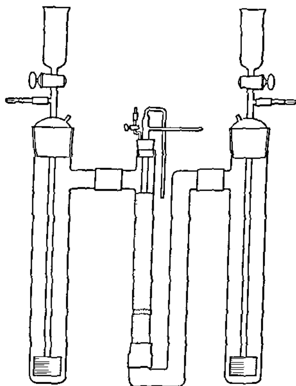


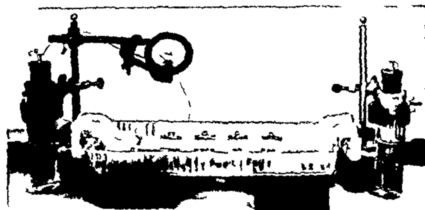
Fig 75 Apparatus for zone electrophoresis

The glass powder does not seem to disturb the separation or the mobility appreciably and it seems to me that this apparatus can be useful for many purposes I believe there is a tendency now in other laboratories as well as in ours to introduce this or similar arrangements in order to have the advantage of complete separation

Once one has decided to introduce immobilizing material it is of course very tempting to try micromethods of electrophoretic separation A particularly attractive and simple technique for doing this is to run the electrophoresis experiments in filter paper as the immobilizing medium That has

been taken up in several laboratories during the last few years probably en couraged to a certain extent by the success of filter paper chromatography which is one of the most striking developments in separation methods during the last few years

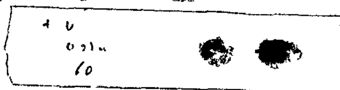
The idea is very simple and the apparatus is shown in Figure 76 A spot of the protein mixture is placed on a filter paper and the buffer solution is allowed to enter the paper which is put between two electrodes With weak currents one need not even have reversible electrodes One may introduce special arrangements to cool the filter paper and it is exceedingly important to prevent all evaporation from different parts of the filter paper In some



F g 76 Apparatus for filter paper electrophoresis

of our experiments we have tried to eliminate that by having the filter paper strip immersed in an organic solvent such as chlorobenzene which does not mix with the buffer solution and does not seem to react with the proteins Otherwise the filter paper has to be enclosed omehow and instead of an organic solvent one may use a well sealed glass chamber After the experi ment has run for a few hours the strip is removed and dried Then it is sprayed just as in chromatography with a suitable dyeing solution which reacts with the proteins We have used for example bromthymol blue which gives a blue color and is a fairly sensitive reagent for proteins

This simple technique gives surprisingly good results but it is essential to use a filter paper with very little or no adsorption We have tried a large number of filter papers and have picked out some which seem to be better than others Still in some papers there are definite adsorption effects which show up as a sort of tail or halo around the spots An idea of the functioning







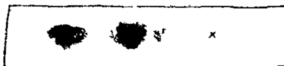


Fig 77 Filter paper electrophoresis diagrams See text for details

of this apparatus can be obtained from Figure 77 which shows some typical diagrams

In the upper strip there is a separation of serum albumin and gamma globulin They have started to the right The other diagrams were made using normal human sera and at the bottom is one using serum from a patient with nephro is

One can carry out rather difficult separations in this way especially if one keeps the protein spot on the piece of paper as long as possible by establishing a countercurrent of the buffer solution through the filter paper This is very easily arranged simply by lifting one side of the apparatus a few centimeters

The method can also be used for quantitative analysis although the filter paper diagrams in Figure 77 demonstrated just a qualitative analysis of course But by cutting the filter paper strip into thin sections a few millimeters wide crosswise to the direction of migration and placing each section in a test tube with suitable colorimetric reagents it is possible to determine the protein con tent in each section and thus obtain a rather good quantitative analysis of the completely separated protein fractions This is microanalysis too in which one can work with less than a milligram of total protein

One has to be careful when drawing conclusions as to mobility from such experiments because one has to know for certain that there is no adsorption As mentioned above in many cases with some filter papers the adsorption effects do not seem to be serious at least not with serum proteins but with some proteins these effects are very marked For that reason of course one must remember that this very simple and attractive technique has definite limitations

Finally I would like to demonstrate another development along the same lines making use of immobilizing media for electrophoretic separations Here we are not concerned with the problem of microseparation but on the contrary with the problem of very large scale separation for preparative pur poses That is a problem which is technically quite difficult because electro phoresis work with very large volumes usually involves work with extremely clumsy apparatus But Dr Svensson and Dr Brattsten in our laboratory have developed a continuous separation technique which is shown in Figure 78

The idea is to combine vertical flow of the solution caused by gravity with horizontal flow caused by the electric field A rectangular trough is filled with glass powder and at the top buffer solution is introduced at an even rate through a large number of inlets Through one inlet in the middle in stead of buffer solution we introduce the protein solution to be separated The protein solution streams evenly downward and if there were no current

flowing the protein would go straight down and out through a few outlets in the middle of the bottom But if a current is sent through horizontally one sees that if a zone contains proteins which migrate in a different way a deflection of the streak of protein is obtained In this case the protein zone has split into two and each of the two will flow out through different outlets at the bottom This process can go on for many hours or even days The protein and buffer solution can run in at the rate of say, 60 ml per hour and

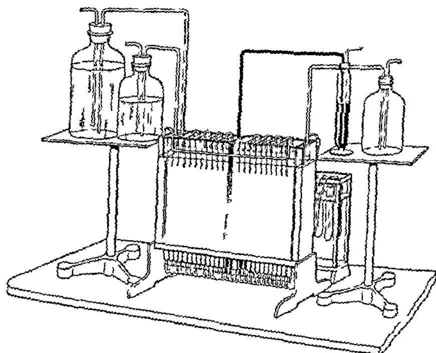


Fig 78 Large scale electrophoretic separatory apparatus

the different fractions are taken out continuously at the bottom This works fairly well in cases where there are big differences in migration between the proteins If there are small differences it is more difficult to obtain good preparations For simple separations it seems to be a very good solution to the problem of working on a preparative scale

CHEMICAL AND NUTRITIONAL DIFFERENCES BETWEEN PROTEINS OF HUMAN AND BOVINE MILK

Professor MELLANDER (Goteborg) Accumulating experimental experience during recent years seems to indicate that distinct differences exist

between the proteins of milk from different mammals In this very short review of the subject I shall talk about casein as I have had some experimental experience of my own with this protein but I do hope that other proteins of milk will also be dealt with in the discussion today

The difference between the caseins of bovine and human milk so far reported have mainly been of a purely chemical nature and rather few and uncertain conclusions concerning their nutritional significance have been drawn

One of the earliest experiences in this field was that of Whoblewski who found in 1894 that during peptic digestion of human casein no precipitate of so-called pseudonuclein was formed but that this did occur when bovine casein was digested That is when we digest bovine casein with pepsin we get a precipitate but when we do the same with human casein no precipitate appears Whoblewski concluded from this experiment that there is an essential difference in structure between the two caseins

In 1913 it was observed by Ylppo that the isoelectric point of the two caseins was not the same and the conclusion was drawn by him that human casein must have more acidic properties After this nothing very important happened concerning the chemistry of casein until Linderstrom Lang and his group published results in 1925 and the following years on the chemistry of cow s casein Among many other things this investigation made it clear that casein from cow s milk is not a homogeneous protein but a mixture of similar proteins differing from each other e g in solubility in pho phorus content and in sensitivity to proteolytic enzymes It was also shown by Svedberg and his collaborators that ca ein from cow s milk is heterogeneous in the ultra centrifuge For casein from human milk no investigation concerning the homogeneity were published until the author s electrophoretic experiments first reported in 1945 I would like to summarize the main results of this work in a few tables and diagrams

In Tables 7 and 8 notice especially the phosphorus content which in cow s casein is around 0 80 per cent In human casein the phosphorus content is much lower just about half that of bovine casein This is the first difference to be noticed between the two caseins

I have no experimental data of my own concerning the amino acid composition of the casein but the results of Williamson published in 1944 demonstrate that both caseins are very similar in amino acid content

Figure 79 shows an *electrophoretic pattern of bovine casein and milk* photographed with the Schlieren technique in 1938 The casein solution is at the left and there are three boundaries called α-casein the fastest moving β-casein and γ-casein the slowest moving In the picture of milk to the

TABLE 7

Elementary Analyses of Bovine Casein (author's preparations)*
(LL = preparation according to Linderstrom Lang
S = preparation according to Sandelin)

Preparation No	Method of preparation	C %	H %	N %	P %	S %	O %	Per cent analyzed
V	LL	53.57	7.13	15.66	0.81	0.64	23.24	101.05
VI	LL	53.08	6.95	15.65	0.85	0.81	22.59	99.93
X	S	53.05	7.05	16.08	0.76	0.88	22.16	99.98
XVI	S	52.90	6.91	15.25	0.76	0.73	22.87	99.42
XVIII	S	—	—	15.05	0.81	—	—	—

* Mellander O *Upsala lakaref forh* 52 114 1947

TABLE 8

Elementary Analyses of Human Casein (author's preparations)*
(E = preparation according to Engel
S = preparation according to Sandelin)

Preparation No	Method of preparation	C %	H %	N %	P %	S %	O %	Per cent analyzed
13	E	53.78	7.12	15.14	0.47	0.65	22.63	99.79
15	E	53.73	7.20	15.11	0.47	0.65	22.31	99.47
19	E	53.73	7.13	14.83	0.47	0.67	22.94	99.77
20	E	—	—	15.16	0.51	—	—	—
36	E	53.50	7.14	15.18	0.46	—	—	—
38	S	53.06	6.97	15.16	0.47	1.13	22.01	98.80
39	S	53.55	7.04	14.89	0.35	1.24	22.53	99.60
40	S	53.17	7.00	14.93	0.32	1.25	22.49	99.16
49†	S	52.47	6.83	14.52	0.42	—	22.98	—
57	S	—	—	—	0.45	—	—	—

* Mellander O *Upsala lakaref forh* 52 114 1947
† Corrected for an ash content of 1.4 per cent

right you can see the same boundaries in the same position but in addition there is another boundary which we believe is due to lactalbumin

The electrophoretic picture of bovine casein using the diagonal slit technique is shown in Figure 80 The α-casein is the largest and the γ casein the smallest component

Figure 81 shows the diagram of human casein with the same technique

and here too there are three different components As a matter of fact human and bovine casein look rather similar by electrophoretic analysis

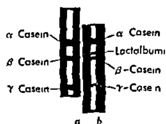


Fig 79 Electrophoretic "Schlieren" photograph of casein (*a*) and milk (*b*)

One difference between the components is that the fastest moving the α fraction has a higher phosphorus content than the γ fraction Human casein shows the same difference in phosphorus content between the different fractions There is a difference between the caseins however in that human casein has a relatively greater amount of the total phosphorus in the α-casein than is the case with bovine casein

Apart from the difference in phosphorus content there are only very slight chemical differences between the two caseins but the behavior of the two

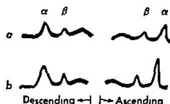


Fig 80 Electrophoretic patterns of bovine casein at different concentrations (*a* 0 5 per cent *b* 1 0 per cent) Phosphate buffer of pH 7 60 and ionic strength 0 15 (phosphate 0 10 + sodium chloride 0 05) Potential gradient about 5 volts per centimeter (Mellander O *Upsala Läk ef forh* 5? 122 1947)

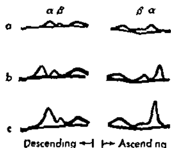


Fig 81 Electrophoretic patterns of human casein at different concentrations (*a* 0 25 per cent *b* 0 5 per cent *c* 1 0 per cent) Phosphate buffer of pH 7 60 and ionic strength 0 15 (phosphate 0 10 + sodium chloride 0 05) Potential gradient about 5 volts per centimeter (Mellander O *Upsala Läk ref fo h* 5? 122 1947)

caseins towards proteolytic enzymes shows very pronounced differences If we follow the change in viscosity when digesting casein with pepsin according to the method first used by Holter Linderstrom Lang and Funder we notice

that when bovine casein is digested we have a steep rise in viscosity after the initial decrease This is not the case when human casein is digested (Fig 82)

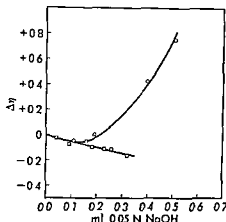


Fig 82 Change in relative viscosity (Δη) during hydrolysis of bovine (O) and human (□) casein with commercial pepsin (Mellander O *Upsala lakaref forh* 52 146 1947)

When following the rate of hydrolysis with formol titration for example we find that bovine casein is much more easily digested than is human casein This was of course a rather unexpected result (Fig 83) The degree of hydrolysis at a given time is much higher for bovine casein than it is for human casein

This experiment was repeated with gastric juice from infants instead of pepsin with the same result (Fig 84) When caseins are digested with trypsin the same difference was demonstrated again (Fig 85)

This difference between the two caseins depends on the formation of enzyme resistant phosphorylated peptides As the resistance of these peptides toward the proteolytic enzymes depends on the presence of the phos

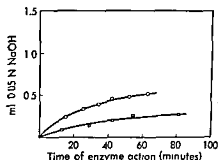


Fig 83 Hydrolysis of bovine (O) and human (□) casein with commercial pepsin (Mellander O *Upsala lakaref forh* 52 143 1947)

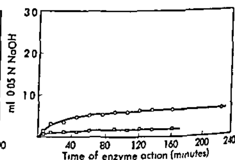


Fig 84 Hydrolysis of bovine (O) and human (□) casein with gastric juice from infants (Mellander O *Upsala lakaref forh* 52 145 1947)

phoric acid in the molecule and as the linkage between the phosphoric acid and the peptide is easily broken by the action of alkaline phosphatase the difference in digestibility can be removed by adding phosphatase to the digestion mixture

The phosphopeptide formed during peptic followed by tryptic digestion are very similar in elementary composition whether formed from bovine or human casein They contain about 6 per cent phosphorus and form very soluble salts with calcium having a calcium content of 10 per cent They also form salts with iron and zinc We have prepared iron phosphopeptides containing about 25 per cent iron and also corresponding zinc peptides Whether these phosphopeptides play a role in nutrition is a question of great importance

A point of special interest is whether there are any differences between such peptides obtained from human casein and from cow s casein Some preliminary experiments indicate that the phosphopeptide from human casein is comparatively more resistant than that from cow s casein to the phosphatases from human intestines

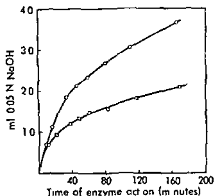


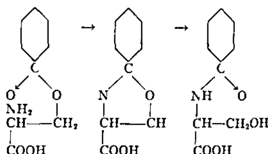
Fig 85 Hydrolysis of bovine (O) and human (□) casein with commercial trypsin (Mellander O *Upsala lak ref fo h* 5? 173 1947)

Some balance and absorption experiments with the calcium salts were discussed at the calcium phosphorus and vitamin D panel and so I shall only repeat that such balance experiments have demonstrated that artificially prepared calcium phosphopeptides from bovine casein can be absorbed from the intestinal tract and used for bone calcification in infants and in rats In experiments on chickens performed according to the method of Olsson there is an antirachitic effect of these calcium phosphopeptides which is significantly higher than the antirachitic effect of the same amount of calcium and phosphorus in inorganic form

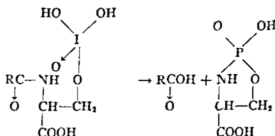
Concerning the amino acid composition of these peptides from cow s and human casein some interesting results have been obtained by Professor Agren s group about which I hope he will tell us a little

Professor LINDERSTROM LANG (Copenhagen) I should like to say a few words about the question of phosphorus and the rennin reaction I don t know whether what I am going to say will interfere with what Professor Agren is about to bring up At any rate it will not have any definite application to the phosphopeptide question

Once Dr Holter and I at the Carlsberg Laboratories were interested in the question of how the phosphorus was bound in casein Claude Rimington who worked in the Carlsberg Laboratories in the thirties indicated the possibility that the phosphoric acid esters with amino acid hydroxyl groups (serine) may behave like the benzoyl esters of serine studied by Bergmann



Reactions of this type would require that the amino group of serine be free which is unlikely since everything points to the fact that serine is bound via its amino group to glutamic acid However the peptide bonds in which the amino groups of hydroxy amino acids are involved are known to be labile probably because of the wandering of the carbonyl group from nitrogen to oxygen (via oxazoline) If the hydroxyl group is esterified with phosphoric acid reactions of the type



may occur which means that paracasein should contain serine linked to phosphoric acid as a terminal group Certain experiments by Holter and Li and by Perlmann seem to support such a mechanism

Professor WALDENSTRÖM (Malmo) I have tried to investigate a few problems of protein metabolism with the aid of electrophoresis and ultracentrifugation in collaboration with Dr Pedersen from the Institute of Biochemistry and Physical Chemistry here in Uppsala and there are a few problems that I would like to discuss very briefly

The question about the form of the gamma globulin peak may possibly be of general biological interest If you make an electrophoretic diagram of serum from a patient with multiple myeloma for instance you always find a very high and very narrow peak usually of gamma globulin It looks something like a church spire But if you investigate the gamma globulins in other conditions e g virus infections you have another type of elevation broad and sloping One might make the assumption that the very high narrow spire could be caused by an increase in one component and the broader type could be caused by an increase in several components

We clinicians have a tendency to exaggerate the uniformity of the gamma globulin I think it is completely wrong and I should like to hear Professor Tiselius opinion on that question To talk about the gamma globulin as we usually do is convenient but we should talk about the gamma globulin family —the gamma globulins It is a whole group of protein components usually containing several functionally different proteins

We find the broader type of curve in many conditions for example in chronic viral infections Pedersen and myself and Dr Sonck from Finland have investigated a number of sera from patients with lymphogranuloma inguinale where an increase in gamma globulin is quite characteristic We have found this broad type in every case The same is also true of cirrhosis of the liver in younger people and in adults and this may be the type of antibody response found in a chronic viral infection It is also possible that other conditions in which the cause of the globulin increase in the serum is not known may be explained as chronic viral infections

When we speak about the nonuniformity of gamma globulin I think it may also be of some interest to remember the ultracentrifugal characteristics of the globulin fraction When you compare a number of sera containing large amounts of gamma globulin you will find that usually the so-called seven component fraction in the ultracentrifuge the one that corresponds to a molecular weight of 150 000 is increased and is identical in size with the gamma globulin fraction the amount of gamma globulin corresponds to the amount of the seven-component fraction

But there are certain exceptions In some cases of multiple myeloma for instance the ordinary sedimentation constant of seven is not found The most important fraction may be one with a sedimentation constant of 11 corresponding to a higher molecular weight In other cases you will find a very high sedimentation constant corresponding to a molecular weight of one million This is usually a gamma globulin but it may be a beta globulin in some instances

I think that this is probably an illustration of the fact that what we call the

gamma globulin is just a group of protein fractions showing the same electrophoretic mobility but otherwise possibly different

I should like to ask Professor Tiselius one other question These very beautiful paper chromatograms seem to afford the chance that clinicians have been longing for to find a very simple method of performing electrophoretic analyses that could be used in clinical laboratories But for quantitative results one needs to dye the paper As there are many dyes that could be used I should like to ask Professor Tiselius what is the relative affinity for the dye of the different protein components Can you be sure that the normal alpha globulin for instance and the pathologically increased alpha globulin have the same affinity for the same dye that you usually use?

I have discussed this problem with Professor Bennhold in Thuringen who is very interested in the transportation of colored materials in serum and the vehicular function of the blood proteins and he has told me that it is a very characteristic feature of different proteins to absorb or carry different dyes How does one control these differences in your quantitative paper electrophoresis studies?

On the glass powder that Professor Tiselius showed us it was possible to get a very good separation of different fractions I should like to hear whether it is possible also to find subfractions For example could you divide the beta-1 and the beta 2 globulins or could you possibly show some inhomogeneity in the gamma fraction?

Professor TISELIUS (Uppsala) These are all very important questions and I shall deal with them in the order in which they were mentioned

I think it should be emphasized very strongly that the homogeneity of a protein component as studied by the electrophoretic technique only means that electrophoretic migration is incapable of further subdividing this component into other components I think that it is true of any fractionation or preparation method that it can only give you information about homogeneity in regard to the particular phenomenon on which the separation is based and in this case it is the charge or the electrophoretic mobility That is true of *all electrophoretically studied protein mixtures and components* Therefore one should always be very careful when stating that proteins are homogeneous if this statement is based upon only one method whatever that may be The electrophoretic method has proved to be very sensitive in many cases but certainly not in all cases And I think it is particularly important to remember for the case of gamma globulin first that the total migration of the gamma globulin is very small You will remember that it appears as the last peak or the last spot in which means that it migrates very slowly course a ich migrates slowly

carries a very low charge and will therefore give very low resolution into its components by electrophoresis So there is a particularly good reason to be careful here and the only thing which is evident from electrophoretic analysis is that the gamma globulin separates fairly well from the rest of the plasma proteins I agree entirely with Professor Waldenstrom that one should be particularly careful and really not speak of gamma globulin but always use the plural gamma globulins

Some experiments have been made by Dr Kirkwood in America with a very ingenious electrophoretic fractionation method It is a sort of electrophoretic convection method to subdivide the gamma globulins and he has obtained some very definite indication that there is a fractionation For the fractionation of the gamma globulins I think that at present precipitation and other methods are necessary and one may hope that at some time in the future chromatography can be applied to proteins also Then the chances of subdividing the gamma globulin into perhaps several distinct proteins might be greater It is also possible that the globulins represent a mixture of continuously varying chemical properties and that there are no really distinct members of the group or that the differences may be so small that it would be extremely difficult to subdivide the group into distinct components by any methods We do not know that yet

The other question dealt with the reactions between dyestuffs and proteins particularly those dyes which are used for staining the paper electrophoresis strips In the first place this technique was used as a qualitative technique just to indicate where the zone is on the filter paper and in that way to get the qualitative information about the number of components present and their migration If one tries to make use of this technique for quantitative purposes (and such experiments have been tried in our laboratory) by extracting the dye afterwards from the spots and estimating the amount of dye by a suitable colorimetric method one runs into the problem which was stressed by Professor Waldenstrom namely that there is every chance that the affinity for the dye is different for example with serum albumin from what it is with serum globulins This has in fact been shown by experiments on separated fractions In the attempts which were first made to evaluate these diagrams quantitatively one had to introduce factors for this variation in dye affinity That is a given quantity of extracted dye would correspond for example to 1 mg of serum albumin and perhaps to 1 7 mg of gamma globulins It is of course not a very rational procedure to have that empirical scale particularly if one has to deal with new proteins formed under pathological conditions for one does not know what factors should be introduced Thus we have tried to improve the situation somewhat by using more rational methods

At present Dr Kunkel is working with a colorimetric method for microprotein determination based on the Folin reagent and the biuret reaction There again of course there must be individual differences between proteins although perhaps the phenomena on which those individual differences are based are easier to define than the factors which influence the combination between a dye and a protein

Of course the ideal method would be to make nitrogen determinations on a microscale and I believe that can be done on these sections of filter paper too One has to work with very small amounts of nitrogen and one may run into certain difficulties because there is always present in filter paper a trace of nitrogen which seems rather erratic and which will influence the results There are also possibilities of doing photometry by ultraviolet light it one can make the filter paper strip transparent by treating it with some suitable solvent. All that will undoubtedly be tried out because we know that a great many research workers are interested in these problems and are trying various means of solving them The method is in principle so simple and requires so little in experimental technique that I believe it is very tempting for many more laboratories to try I think too that it gives many opportunities for ingenuity in finding further improvements

The glass powder column used in the last apparatus I described is of course more rational from this point of view because the protein is in solution after the experiment and you can work with somewhat larger amounts There the ordinary micro Kjehldahl determinations can be used So far however the degree of resolution of this method or any of these methods is not quite comparable to what you obtain by the boundary electrophoresis technique with optical observation I think that is chiefly a technical problem I cannot see any reason why one should not be able to attain the same degree of resolution by methods with which one works in columns or immobilizing media of some sort

Professor MELLANDER (Goteborg) When using electrophoresis in pediatric research it is important to be able to obtain results with very small amounts of plasma What are the limitations in this respect?

Professor TISELIUS (Uppsala) To use very small amounts of proteins in *free* electrophoresis is difficult It is necessary either to use low concentrat ons with consequent decreased stability of the boundaries or to use rather narrow tubes which also present many difficulties No doubt however improvements in the optical system will make it possible to study the free electrophoresis of dilute solutions better than we can do now For many purposes where microdeterminations are essential filter paper electrophoresis is at present the most convenient method

Professor AGREN (Uppsala) In connection with Dr Mellander s in troductory talk on phosphopeptones I would like to mention some chemical investigations which I have carried out on his preparations in collaboration with Dr de Verider and Mr Glomset A comparison was made between

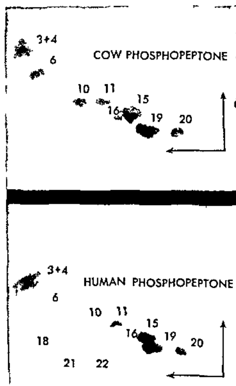


Fig 86 Photograph of a two-dimensional (phenol/pyridine amyl alcohol) chromatogram showing the positions of the amino acids in hydrolysates of cow phosphopeptone and human phosphopeptone *1* tyrosine *3* isoleucine *4* l ucine *6* val ne *10* alan ne *11* threonine *15* serine *16* glyc ne *18* histidine *19* glutamic acid *20* aspartic ac d *21* arginine *22* lysine

phosphopeptones prepared by Mellander from bovine casein human casein and goat s milk casein The results are given as one and two-dimensional paper chromatograms of the hydrolyzed phosphopeptones (Figs 86 87 88)

If one assumes that one molecule of each amino acid is present in the phos phopeptone molecule this would correspond to a molecular weight of about

1000 Obviously the phosphopeptones prepared according to Mellander's method are of higher molecular weight than those obtained by some other authors This fact may be associated with the low phosphatase activity of the trypsin used by Mellander

The presence of threonine in the phosphopeptone preparations was of

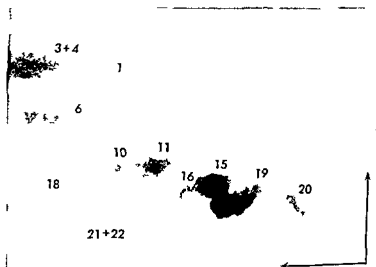


Fig 87 Photograph of a two-dimensional (phenol/pyridine amyl alcohol) chromatogram showing the positions of the amino acids in a hydrolysate of goat phosphopeptone

special interest Up to the present it has been assumed that serine is the only hydroxyamino acid of casein which is esterified with phosphoric acid Lipmann in 1933 prepared an amorphous silver salt of serine phosphate from casein following hydrolysis by boiling with 2 5 N hydrochloric acid In his paper Lipmann gives the following analytical figures for the silver salt

Percentage	C	H	N	P	Ag
Calculated	7 12	0 99	2 76	6 12	63 8
Found	7 56	1 24	2 79	5 65	61 2

Phosphothreonine is about as resistant to acid hydrolysis as phosphoserine (Plimmer 1941) and in our view the possibility that small amounts of phosphothreonine might be present both in such a preparation and in phosphopeptone could not be excluded

Some of our interest with regard to Mellander's phosphopeptone has been

focused upon the possibility of separating the preparation into subfractions with the idea of obtaining a threonine rich phosphopeptone Only preparations from bovine have been used

The original preparation of phospho peptone in the form of its calcium salt contained glutamic acid aspartic acid serine glycine alanine threonine valine leucine and isoleucine with traces of proline and the basic amino acids (cf Figs 86–88) When this preparation was subjected to electrodialysis the basic amino acids were removed and the phosphopeptone was trans formed into the free acid

When small quantities of the calcium free preparation were put on paper strips and these developed with alcohol water mixtures it was found that a separation into two fractions could be attained with 90 per cent alcohol one fraction was soluble and fol lowed the solvent front and one was in soluble and remained on the base line Utilizing this fact a rough separation was first carried out by adding absolute ethanol to a water solution of phosphopeptone to a concentration of 90 per cent This caused the precipitation of about three fourths of the solute and on adding ether to the filtrate the remaining one fourth was pre cipitated out of solution When these two fractions were run on columns packed with paper powder with 90 per cent ethanol as the eluting solvent a still further separation could be obtained into relatively soluble fractions (I and III) and relatively insoluble fractions (II and IV) (See Table 9)



Fig 88 Photograph of a one dimensional (tertiary amyl alcohol in di ethylamine atmos phere) chromato gram showing the positions of the amino acids in hy drolysates of cow pho phopeptone (C) and human phospho peptone (H)

Working with fraction IV which was available in relatively large quantities it was possible to obtain still another separation again using paper chromatograms followed up on a preparative scale with paper powder columns This time

fractionation was carried out using 70 per cent acetone as solvent (IV A and IV B)

Our experiments up to now indicate that far from being a single compound, our preparation is made up of several molecules ranging from 10 to 17 amino acids per peptide Moreover when dinitrophenyl derivatives of these fractions were hydrolyzed several yellow spots appeared in each case so that it appears that even our several fractions are very complex

With regard to the amino acid content of the different subfractions we have been able to separate a phosphopeptone free from glycine and threonine

TABLE 9
Table of Data for Fractions from Mellander s Phosphopeptone Preparation

Fraction	Approx no amino acids per peptide	Approx no phosphate groups per peptide
Whole preparation	10	2 4
Ether ppt	10	2 6
Fract I	10 5	2 7
Fract II	12	4 1
ROH ppt	12	2 95
Fract III	12 5	3 5
Fract IV	14	3 2
Fract IV A	17	4 2
Fract IV B		

(IV A) A fraction has also been obtained containing more threonine than the original preparation So far we have not been able to isolate threonine phosphoric acid but our investigations in this direction are being continued

In discussing how phosphoric acid is bound to serine Professor Linderstrom Lang told us that this question is not yet definitely settled In 1927 Rimington found some differences in the general behavior of the esterified phosphoric acid groups of phosphopeptone Two thirds of the total phosphorus was removed by bone phosphatas of phosphoric acid the remaining one third ver, alm also in ester linkage as it was removed by terase o acts The explanation given by Rimington molec oric acid were united through one hydrox o the ain while the third formed er link

Professor LEVINE (New York) There is no question but that fundamental studies of the nature that we have heard about this morning will be necessary to advance in the future our knowledge of nutritional problems in infants Without any question methods utilizing electrophoretic technique paper chromatography and tracer substances will be the methods of the future Those of us who have dealt with purely analytical chemical methods can merely attempt to compare the results attained by those methods with some of the implications which were brought out today

Professor Mellander pointed out that the casein in cow s milk is more easily digested by proteolytic enzymes than the casein from human milk Professor Stearns indicated that a ceiling for protein digestion and utilization is not attained by infants until they reach an age of about 3 to 3 1/3 years These observations I think are confirmed by some of the studies made by my colleagues Dr Gordon and Miss McNamara on the absorption and retention of proteins by premature infants In these balance studies prematures received isocaloric feedings containing equivalent protein intakes in the form of human and cow s milk The intake was about 2 8 gm of protein per kilogram of body weight per day and on the human milk the retention was 0 25 gm of nitrogen per kilogram and on the cow s milk 0 275 gm per kilogram indicating that the absorption and utilization and retention of protein were equivalent whether the infants received the protein in the form of cow s milk or human milk When the protein intake was increased in the form of cow s milk from 2 8 gm per kilogram to 5 gm per kilogram and in one instance to 9 gm per kilogram a very high intake the retentions continued to rise so that on the 5 gm per kilogram the retention was 0 35 gm of nitrogen per kilogram and on the 9 gm of intake it was 0 45 gm of nitrogen This indicated that there is no ceiling apparently in the premature infants at least at the level given and that they are just as capable of absorbing protein when given in high quantities as full term and older infants

In view of the fact that until several years ago it was considered that human milk was biologically superior to cow s milk because of the sulphur-containing proteins it would be interesting I think to apply some of the techniques used by Professor Mellander to study the lactalbumin in human and cow s milk because of the marked difference in the cystine content of the two lactalbumins Since methionine has been shown to be an indispensable amino acid and since it is present in high concentration in cow s milk and human milk the techniques applied by Professor Mellander may indicate perhaps a different degree of absorption and utilization of the cystine of bovine and human lactalbumin This I realize is bringing the discussion down to a far simpler level but after all this is a seminar on infant metabolism!

Professor MELLANDER (Goteborg) I would like to ask if the output of amino acid and peptide nitrogen was determined in the balance experiments of Dr Levine or just the total nitrogen This is of importance for if much of the nitrogen excreted is amino acid or peptide nitrogen this means that the protein given is not fully utilized

Professor LEVINE (New York) Obviously this is the critical point Only total nitrogen was determined in the urine There are observations with human and cow s milk in which cow s milk containing a far higher protein content was given to premature infants and in which there was an excretion of phenylalanine and tyrosine in the form of the aromatic organic acids parahydroxyphenylpyruvic and parahydroxyphenyllactic acids That did not occur when the infants received human or cow s milk and lower protein intakes So that apparently only when a certain level of protein is reached is the premature unable to break down completely certain of the amino acids to urea excreting them instead in the form of intermediates of the amino acids Parenthetically it is interesting that when vitamin C is given this defect disappears But I can t answer your specific question

Professor BESSEY (Chicago) The nature of the preparation of the phosphorus-containing peptides is not clear to me What is the general nature of those preparations? Do they come from partial hydrolysis of casein? Are they present in milk as peptides?

Professor MELLANDER (Goteborg) They are not present in milk but I can tell you how to prepare them Digest a solution of casein with pepsin and continue the digestion with trypsin which is relatively free of phosphatase When the tryptic digestion is finished you can precipitate the peptide as for example the calcium salt

Professor BESSEY (Chicago) Then I am not quite clear As I understood it trypsin is capable of hydrolyzing phosphates from such compounds Did I misunderstand?

Professor LINDERSTRÖM LANG (Copenhagen) That is not the case The trypsin is not able to split off the phosphorus in casein

Professor BESSEY (Chicago) How much phosphate if any is split free as inorganic phosphate as a result of the peptic digestion? Do you lose some?

Professor LINDERSTRÖM LANG (Copenhagen) I lose some When you digest human casein you get, if you are lucky about 10 per cent of phosphorus turned over

Professor VAHLQUIST (Uppsala) I should like to ask a question about the nature of the protein split product which is *absorbed from* the intestine We have heard from Professor Ågren that the phosphopeptide de

scribed by Dr Mellander is a fairly complicated product and you might question whether such a substance can be absorbed without further splitting This raises the question of whether unsplit proteins can be absorbed from the intestine There is a possibility of studying this problem by using antibodies which are in a way labeled proteins It was found several years ago that newborn calves absorb large quantities of specific antibody proteins from the intestine According to recent investigations by Phillips and Hansen the capacity to absorb antibodies is restricted to the first few days of life

An experiment of my own may clarify this A cow had some 40 units of diphtheria antitoxin in the blood The calf at birth had no detectable antitoxin i e there had been no passage through the placenta After having received half a liter of the cow s colostrum in 6 to 8 hours the calf had a titer of 17 units Evidently there was absorption of products which had antibody activity

If you give the same cow s colostrum even in tremendous amounts to newborn infants you find no antibody in the serum If you go a step further and give human serum to newborn children you will find at the most traces of antibody absorbed Although it is very hard to get human colostrum rich in diphtheritic antibodies we have been able to obtain enough to perform a few experiments They indicate that homologous colostral antibodies are absorbed very little if at all I stress this especially because there seems to be a very selective process in the absorption of antibody proteins in the calf If you give the calf his mother s serum instead of colostrum he will absorb practically nothing

Professor LEVINE (New York) Many years ago Professor Schloss in New York showed that when native cow s milk proteins were given to newborn or marasmic infants antibodies appeared in their serum which could be identified by passive transfer and skin tests That capacity to absorb native protein however disappeared fairly rapidly

Professor WALDENSTRÖM (Malmo) Let me take an example from adult medicine where I feel more at home and which may be interesting for pediatricians Vahlquist discussed the problem of the absorption of the intact protein molecule In many instances we can assume that it is broken down into smaller particles though not to amino acids The question arises, what is the size of a molecule that can pass through the membrane of the intestine? Recent experiments performed on vitamin B_{12} seem to be of interest in this connection

If you give vitamin B_{12} perorally to an adult with pernicious anemia you will get no response if you do not give very large doses Practically no B_{12} seems to be absorbed through the intestinal mucosa of such a patient But if

you add normal gastric juice you will get a rapid and complete response on small doses of vitamin B_{12} These experiments were first performed at the Mayo Clinic and have been confirmed elsewhere There are several possible theoretical explanations of this fact One is that you break down the vitamin B_{12} molecule which does not seem very probable Another is that you favor the absorption of this molecule and I suppose that this is really correct If this is the case it would show that certain factors may favor the passage of quite large molecules (1200–1500 molecular weight) through the intestinal membrane of the adult

Professor RÄIHÄ (Helsinki) I have some observations on nitrogen retention in children 2 to 4 months of age which are of some interest in connection with Professor Stearns s talk

We performed experiments with a constant protein diet with carbohydrates and fats added for 10-day periods We had 10 days with a carbohydrate rich diet and then 10 days with a fat rich diet with enough carbohydrates to prevent acidosis The nitrogen retention was about twice as high during the carbohydrate rich period as it was during the fat rich period

Professor STEARNS (Iowa City) We have observed with older children that the weight gain and nitrogen retention both were far higher with high carbohydrate low fat diets The weight gain was lower with the same protein intake when a larger percentage of the calories were given as fat

Professor AGREN (Uppsala) The time factor of absorption may be of interest in this connection All amino acids must be available at the same time for absorption Melnick and Oser recently reported that in food products improved by heat processing and exhibiting no change in amino acid composition or degree of digestibility the rate of enzymatic digestion is critical For example methionine in raw soybean meal was absorbed so late during the gastrointestinal journey that it failed to supplement the remaining amino acids At a result inefficient utilization occurred

A similar mechanism could possibly explain some of the large differences in utilization of human and cow s milk found by French authors and also reported by Dr Jonxis in Leyden

Professor LINDERSTRÖM LANG (Copenhagen) What is the role of bile in the digestion of the calf? Bile is exceedingly effective in denaturing proteins in the intestinal tract and possibly it is those proteins which are denatured that will not be absorbed Is it known whether there is less bile secretion in the first two days of life?

Professor VAHLQUIST (Uppsala) I cannot answer this question Two possibilities may be discussed Either the antibody protein is not split normally in the calf's first two days or the process of absorption itself is differ

ent This is really fundamental and it is amazing that nobody has tried to solve it so far

CREATINE AND CREATININE METABOLISM IN INFANCY

Professor STEARNS (Iowa City) Figure 89 shows the relationship between urinary creatinine and the weight of the baby The excretion of creatinine is a function of weight with a correlation of 0 9 which is unusually high for biological data and particularly with such a wide range of data

Figure 90 shows the creatine in the same way The correlation coefficient here is about 0 8 The individual variation in creatine was much more marked than in creatinine A few infants showed regularly more creatine than creatinine but the majority showed consistently more creatinine than creatine A very few babies started in early infancy with a higher creatine than creatinine output gradually increasing the latter until they reached the more common relationship It was more common however for an infant to keep the relationship between creatine and creatinine which he had attained by the second month

Figure 91 shows creatinine and creatine excretion per kilogram of body weight At birth the creatinine averaged 10 mg per kilogram and in these babies fed a formula of undiluted cow s milk the creatinine rose very sharply so that in two or three weeks it attained the mean value of 12 5 mg per kilogram where it stayed through infancy Thus these babies showed what Scammon has reported that muscle content tends to remain a constant proportion of body weight throughout infancy In breast fed infants the creatinine averages 10 mg per kilogram so that the excretion at birth is probably maintained by the breast fed infant If the infant is given a higher protein diet the amount of musculature very quickly rises to a maximum which differs somewhat for each infant but which that infant tends to maintain throughout infancy The creatine in contradistinction to the creatinine is only about 5 mg per kilogram at birth When the babies are given the higher protein diet it rises much more slowly than the creatinine and reaches a constant value at about 3 months of age

Figure 92 relates daily creatinine excretion to body weight for children up to 12 years The relationship between the amount of creatinine excreted and body weight is maintained after infancy until the child weighs between 12 and 13 kg Then there seems to be a sharp increase in the rate of excretion of creatinine which we would interpret as a sharp increase in the rate of growth of the musculature in relation to the body as a whole

Figure 93 shows creatinine excretion per kilogram of body weight against

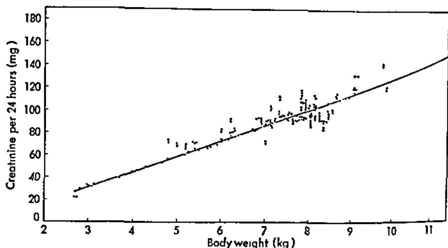


Fig 89 Relationship between creatinine excretion (in milligrams per 24 hours) and body weight (in kilograms) during infancy (Catherwood R and Stearns G *J Biol Chem* 119 201 1937)

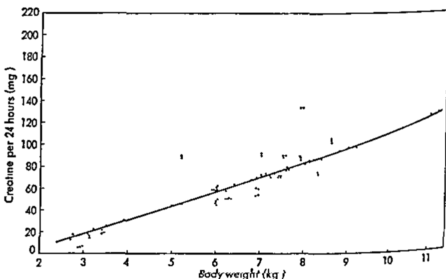


Fig 90 Relationship between creatine excretion (in milligrams per 24 hours) and body weight (in kilograms) during infancy (Catherwood R and Stearns, G *J Biol Chem* 119 206 1937)

age The data for infancy are not all included but the line through the mean is shown at 12 5 mg per kilogram and is constant showing that the muscle is growing at the same rate as the whole body until the child is about a year and a half of age Then there is a very wide scatter between 1½ and 3

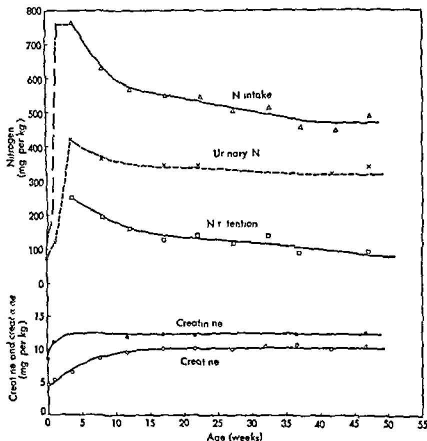


Fig 91 Creat nine and creatine excretion (in m lligrams per kilogram) in relation to age of the infant and to n trogen int ke and retention (Catherwood R., and Stearns G *J B ol Chem* 11) 08 1937)

years with a tendency to settle at a higher rate of creatinine excretion with a mean closer to 20 mg per kilogram by 4 years of age and a small later increase All of these data were from children given an ample intake of protein The data at the far ri ht of the curve were taken from a study of older adolescents in the literature

Creatinine per 24 hours (mg)

Body weight (kg)

Fig 92 Urinary creatinine per 24 hours in relation to body weight of children between birth and 12 years of age

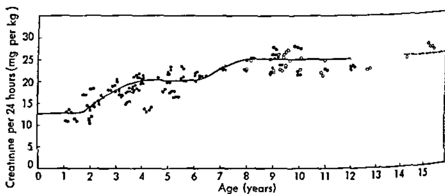


Fig 93 Urinary creatinine (in milligrams per kilogram per 24 hours) in relation to age

In children whose weight and height are within the normal range but whose nutrition has been somewhat substandard except for calories there are some what lower creatinine values Apparently the child makes what muscle he can with the amount of protein given to him Often children of 12 excrete only 18 to 20 mg creatinine per kilogram Children with rheumatic fever who are at bed rest but given a very high protein diet have a still lower cre atinine excretion per kilogram than the poorly fed children who are up and about And children with muscular dystrophy of course will show still less creatinine excretion It has been our observation that children with mus

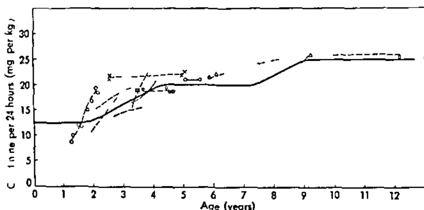


Fig 94 Creatinine excretion (in milligrams per kilogram per 24 hours) of young children stud ed for many months consecutively and of older ch ldren studied at intervals These data show that muscle grows faster than the body as a whole during the period 18 months to 4 years of age

cular dystrophy who excrete 10 mg or more of creatinine per kilogram are usually able to walk though they may not be able to get up from the floor without help With a creatinine excretion of 5 mg or less per kilogram the child is always completely helpless The creatinine excretion per kilogram measured at intervals is a good index to the rate of deterioration of a child with this disturbance We studied one child who was born without legs but with buttock muscles and with only the upper half of the humerus and the houlder girdle muscles His creatinine excretion agreed closely with the *amount predicted from his muscle mass We feel that the creatinine excre* tion per 24 hours is a very close measure of the total skeletal musculature of the child

Returning to the wide variability that we found around 2 to 3 years of age Figure 94 will show you data from young children we were able to study over

periods of up to 1¼ years in length In each child there is a sharp increase in creatinine excretion during the runabout years Each child seemed to show a rate of increase characteristic of his own body in that the slope of the curve was different for each child Some children attained a level excretion by 2½ years of age but others did not until nearly 4 years of age But in all of the children whom we have studied we have found this very sharp rate of change in creatinine which we have interpreted as indicating a much more rapid growth of skeletal musculature than of the body as a whole The reason for this change appears to be the habitual assumption of the upright posture and the need for a greater quantity of musculature to hold the body erect This is especially true when the long bones are growing rapidly

I do not know what your feeding habits are in Sweden but in America it is not common unfortunately for the child of this age to be given a high protein diet He is a slow eater and does not chew very well So the family compromises on cereals and soft foods and most children coming to us in the hospital show a creatinine excretion lower than we think desirable When a higher protein intake is given creatinine excretion rises very quickly We find throughout childhood that any child whose creatinine excretion is lower than we think desirable will retain nitrogen very quickly if he is given an adequate intake That is not always true incidentally of calcium and phosphorus but it has been true of protein in that the higher the protein intake the higher the retention will be We have had children of average weight for height gain apparently 20 per cent in the quantity of musculature in three months without any weight gain They have apparently substituted muscle for fat or water The child is a different child clinically he is more alert and has much more energy Therefore it is our feeling that a higher protein intake than is commonly fed is probably wise during the period of growth and that a high protein intake is particularly desirable up to the age of three so that the child can have sufficient protein to manufacture the quantity of muscle necessary and desirable in order to maintain good posture

GAMMA GLOBULIN AND ANTIBODY RESPONSE

Dr ÖBERG (Uppsala) Our knowledge of the influence of dietetic factors on resistance to infection is too scanty to justify general conclusions Many investigations have shown however a lowering of resistance if dietary deficiencies are of such a degree that they lead to a manifest avitaminosis or an extreme depletion of the blood proteins But we know little about the problem of whether qualitative changes in dietary proteins can influence antibody formation The superiority of breast milk in feeding full term

infants in the first six months seems to be generally accepted There is not however as great a difference in morbidity between artificially fed infants and breast fed infants as formerly and if there is a difference in resistance to infection its interpretation is unclear

The method of transporting antibodies from mother to offspring varies in different animals probably with the structure of the placenta We know for example that the calf receives antibodies from the cow via the colostrum during its first few days In humans the transfer of antibodies from mother to offspring probably occurs through the placenta and in a lesser degree or not at all through colostrum or milk Even if breast milk is not a source of antibodies for the infant it can be of importance in creating resistance against infection in other ways

First of all optimal nutrition may favorably affect effective antibody response in infection or during active immunization It may also be possible that in the synthesis of antibodies certain proteins are more valuable than others depending on the nature and number of the amino acids in antibody and protein The question then arises Is there any difference in antibody response between suckling and artificially fed infants or animals?

In order to study this question Professor Mellander and I have worked in part with lambs One of a pair of lamb twins was breast fed by its mother and the other was fed on an artificial diet This diet was prepared from a whole cow s milk powder fortified with fat and proteins to about the same proportions as sheep milk When the lambs were about one to two weeks old they were inoculated intraperitoneally with a chicken adapted influenza virus Blood samples were taken before inoculation and several times later for one to two months We determined the antibody titer with an agglutination inhibition test The serum proteins were determined electrophoretically For the electrophoresis we have used a Tiselius microapparatus from the Perkin Elmer Corporation which permits a determination of 0 5 to 1 0 ml serum

The results are given in Tables 10 and 11 Higher antibody titers and γ globulin levels were obtained in the suckling than in the artificially fed lambs These are preliminary results but justify further investigation along these lines

Professor WALDENSTRÖM (Malmo) In a patient who had just recovered from severe typhoid fever and malnutrition serum proteins were measured by salt precipitation methods electrophoresis and ultracentrifugation Both H and O agglutinins were also determined Immediately after the disease the patient had a total serum protein of 2 5 gm per 100 ml with extremely low albumin gamma globulins and agglutinin titers When his

TABLE 10

Antibody Response to Influenza Virus Inoculation in Suckling and Artificially Fed Pairs of Twin Lambs

L 21 (suckling) — Born March 19th, inoculated March 24th — L 22 (artificially fed)

Date	Antibody titer	Serum protein gm /100 ml	γ globulin gm /100 ml	Antibody titer	Serum protein gm /100 ml	γ globulin gm /100 ml
24/3	0	6 46	3 23	0	6 0	3 9
4/4	512	6 26	2 42	1024	5 65	1 78
13/4	2048	6 63	2 45	1024	5 41	1 06
20/4	4096	5 95	1 68	512	5 26	0 94
Mean after inoculation	2219	6 28	2 15	853	5 44	1 36

L 13 (suckling) — Born March 9th, inoculated March 24th — L 12 (artificially fed)

Date	Antibody titer	Serum protein gm /100 ml	γ globulin gm /100 ml	Antibody titer	Serum protein gm /100 ml	γ globulin gm /100 ml
24/3	0	5 33	0 84	0	5 39	0 96
4/4	512	5 30	0 57	512	5 40	0 57
13/4	128	5 60	1 16	256	6 55	0 66
20/4	256	5 50	0 75	128	7 51	0 84
Mean after inoculation	300	5 57	0 83	300	6 48	0 69

L 11 (suckling) — Born March 8th, inoculated March 21st — L 10 (artificially fed)

Date	Antibody titer	Serum protein gm /100 ml	γ globulin gm /100 ml	Antibody titer	Serum protein gm /100 ml	γ globulin gm /100 ml
21/3	0	6 97	1 68	0	6 23	0 37
4/4	1024	6 05	0 78	512	5 94	0 72
13/4	512	5 67	1 56	256	4 40	0 43
20/4	4096	6 18	1 28	256	5 77	0 75
Mean after inoculation	1877	5 53	1 19	343	5 33	0 63

condition improved and he began to eat total serum protein gamma globulins and typhoid agglutinins rose rapidly

I think that possibly this is an illustration of the fact that when the synthesis of serum proteins suffers during a time of depletion of amino acids in the food the production of specific immunological substances is also defective

TABLE 11

Antibody Response to Influenza Virus Inoculation in Suckling and Artificially Fed Twin Lambs

Mean values after inoculation of influenza virus	Antibody titer	γ globulin gm /100 ml
Suckling lambs	1465	1 36
Artificially fed lambs	500	0 89

I would not venture to state that *this* is the same as lowered resistance but it is an indication of connection between the function of gamma globulins and of certain antibodies

Professor VAHLQUIST (Uppsala) This raises an *important* question i e the relationship between gamma globulin content and content of antibody measured serologically This is a very intricate problem I think I am right in saying that normally only a *small part* of the gamma globulin *fraction is* of antibody character Hence the fact that the gamma globulin fraction *measured chemically or electrophoretically is low does not necessarily mean* that the titer of a specific antibody could not be comparatively high You certainly find of course that with a very high titer of pneumococcal antibodies you may have an increased gamma globulin level and *in* that case evidently antibody titer is reflected in the chemical level But I imagine you can have an increased titer of antibody without necessarily having increased gamma globulin

Professor LEVINE (New York) What is the structure of the placenta in the sheep?

Dr OBERG (Uppsala) The structure of the placenta in the sheep is the same as in the cow The lambs *in other words* get antibodies through *colostrum or milk* But *if there is a difference in antibody* response on active immunization between suckling and artifically fed lambs this *cannot depend* on the transfer of passive immunity from mother to *offspring* The diet can however be of importance for antibody formation after active immunization

Professor LINDERSTROM LANG (Copenhagen) Albert Fisher has been doing some experiments on tissue culture cells in which he finds that half broken-down proteins of the homologous type are very good substrates for growth of cell Half broken-down proteins of heterogenous tissue are very much poorer than peptides of the same group This may have relevance to the point made in the discussion this morning that far larger portions of proteins than we have believed may under certain conditions be taken up

without being broken down They may be useful in the building up of cells and probably also in building up the system to make antibodies

Professor VAHLQUIST (Uppsala) May I add a word to the discussion concerning the relationship between gamma globulins and serologically determined antibodies? The conditions in the fetus offer a special opportunity to study this problem

Moore Depahn and collaborators at Columbia in New York have measured electrophoretically the composition of the serum at various ages of gestation That is in fetuses from abortions or in premature children they measured the serum concentration of various protein components At the middle of gestation the gamma globulin measured in this way is very low and then there is a steady rise until delivery At delivery the amount of gamma globulin is somewhat above the level of the mother but after birth there is a decline for the first two or three months and then a fairly stable level is reached

I have examined the titers of antistaphylolysin and antistreptolysin in three groups of children These consisted of full term infants premature infants and fetuses from legal abortions The mother and full term child had about the same titers at birth of both antistaphylolysin and antistreptolysin In premature infants antistaphylolysin was much the same in mother and child whereas the antistreptolysin appeared to be lower in the child At the middle of gestation at which period these legal abortions were performed only 3 out of 24 fetuses showed any measurable titer of antistreptolysin and none had any measurable titer of the antistaphylolysin These two types of antibodies appeared not to have passed over to the fetus at the middle of gestation whereas between the middle of gestation and up to the time of premature birth there are successively greater amounts transferred across the placenta so that the concentration in the premature infant s serum is not too different from that of the mother The behavior of the titers of these two types of antibodies corresponds to the findings of Moore Depahn and co workers concerning the gamma globulin fractions

I have a few observations on diphtheria antitoxin which bear out the same result And I have heard from one American colleague that he has found the same behavior in the case of diphtheria antitoxin It is not surprising that this should be so since the placenta undergoes very marked changes in structure during the period after the middle of gestation with the number of villi increasing tremendously so that the contact surface between the blood systems of mother and fetus increases greatly

Professor LEVINE (New York) The amino acid compositions of human and cow s milk have been determined chemically by Block and

Bowling and the differences were principally in the cystine and methionine content I do not know whether lamb s milk has been analyzed It might be interesting to see whether the proportions as well as the total amino acid content are similar to cow s or human milk in order to determine whether the differences in the synthesis of homologous proteins are related to the composition of milk I wonder whether there is any knowledge on that point?

Professor MELLANDER (Goteborg) I don t know of any amino acid analysis of the milk of lambs but even if you have two proteins of the same amino acid composition it is still not certain that the peptides formed during digestion have the same biological properties Among other things the order in which the amino acids are linked together may vary

Professor VAHLQUIST (Uppsala) The question of the transfer of proteins from mother to fetus in human beings in utero is very important but rather complicated At least part of the gamma globulin found in the serum of a newborn child must have come from the mother if one judges from available evidence The decline in concentration following birth is evidently due to a disappearance without new gamma globulin being formed A question of great interest is at what age during gestation or immediately following birth active formation of gamma globulin starts

If you immunize a newborn child with a good antigen you find that he reacts to this antigenic stimulus provided that there is not a high concentration of antibodies in the blood at birth The reaction however is delayed In an older child or in an adult four weeks following injection of diphtheria toxoid there is a measurable titer of antibodies in the blood In newborns more than half show no antibodies at all four weeks after injection But three months after injection all the newborn full term children had formed antibodies and the final titer was not markedly lower than that reached in older infants or in adults There seemed to be a slower response to this antigenic stimulus in the newborn I do not believe that is true just for diphtheria toxoid Analogous delays occur in response to BCG vaccination and the tuberculin reaction I think much work needs to be done on the capacity of the child to form proteins

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CHAPTER IV

Panel on Vitamins

CAROTENE AND VITAMIN A REQUIREMENTS IN INFANTS

Professor STEARNS (Iowa City) Plasma vitamin A in its relation to the intake of the infant has been studied by various people It is usually stated that the plasma vitamin A level of persons is very largely independent of the immediate intake of vitamin A or carotene and is greater in persons of higher than in persons of lower economic status

The plasma vitamin A level of the newborn infant is considerably lower than that of the mother and is stated to rise sharply on the fourth day of life or at the time when feeding begins It is known that the fetus stores vitamin A in the liver and that storage increases toward term Apparently the plasma vitamin A level *is not dependent on the amount stored* in the liver

Data in the literature show that young infants who are getting an adequate intake of vitamin A can be given very large additional amounts without any marked change in the plasma level A decline of the plasma vitamin A is usually considered as evidence that the liver stores are exhausted Still less is known about carotene levels than about vitamin A levels in infants It appeared to us that it would be useful to obtain some data on plasma levels from infants who were being given a constant intake of vitamin A daily throughout the period of infancy

After several years of study we had evolved a semisynthetic diet to which all of the vitamins were added separately It was planned to use this type of dietary regimen in the study of the requirements of various vitamins

Unfortunately for the vitamin A investigation we started with the D group of vitamins and were offered a grant of money from a commercial firm if we would use the source of vitamin D which they manufactured It was good, but it contained no vitamin A We explained that we had made our original tests with a source containing both vitamins A and D and we were told they

would obtain vitamin A for us We knew the source and knew it was good since vitamin A from that source had been used by others for vitamin A studies So we were satisfied without testing it

But I think that the particular material which was sent us was a little too concentrated and did not disperse as well as it appeared to At least after

Fig 95. Slowing of growth of baby R T The arrow marks the time of introduction of a more water miscible vitamin A preparation in the diet.

the study had run for some three to four months we found that the three oldest infants showed a very definite slowing of growth Since we were concerned with the water soluble vitamins we tried increasing the amount of those vitamins which we thought might be at fault but to no avail

We had been concerned about the dispersion of our vitamin A and were waiting for another product It arrived about this time and we gave it to one infant merely as a control and this infant showed a sharp rise in rate of growth So we changed to this source of vitamin A for all the infants Un

fortunately we have no plasma vitamin A levels during this period of slow growth

Figures 95 and 96 show the rate of growth of these babies none of whom showed skin lesions or Bitot s spots The resumption of growth was very prompt after the administration of vitamin A

Fig 96 *Slowing of growth and weight of baby M A corrected by substitution of more dispersible form of vitamin A for a poorly dispersed form The arrow marks the age at time of substitution*

We started a serious plasma A study the next year Groups of babies were given three different amounts of vitamin A One group received 750 units of vitamin A either as the palmitate or as the acetate if they were getting th synthetic diet or from partially skimmed milk if the latter was their diet The milk fed infants got only the carotene of the milk while the infants receiving the synthetic diet received carotene in orange juice Thus the carotene intakes were similarly low A second group was given about 1500

units of vitamin A daily either as milk or as synthetic A and a third group was given about 2500 units

Figure 97 shows the data obtained on serum A and on carotene

The first group receiving 750 units of vitamin A is the most important These babies *ranged from 3 to 15 weeks of age* Most of *them* showed plasma vitamin A levels between 20 and 30 μgm per 100 ml and the mean value was slightly above 20 μgm There was little difference from infant t

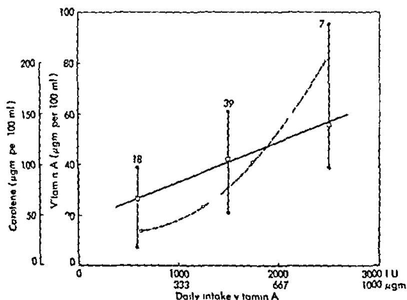


Fig 97 Plasma values for vitamin A and carotene in relat on to *daily intake* The squ res repre ent the mean values for pl ma A observed at e h level of intake the perpendicular lines show the range of values observed The numbers above e ch represent the number of values recorded for e ch group The circles and d sh l ne represent pla ma carotene levels.

infant although this group included infants whom we expected *from* their maternal history to have the best liver storage of A as well as infants in whom we expected to find the least storage

The infants getting 1500 units of vitamin A daily showed definitely higher values and the mean value 40 μgm per 100 ml approximated the maximum *value obtained in the first group* *In this second group the plasma vitamin A* level tended to vary with the individual as has been reported previously

The infants in the third group all over 6 months of age received between 2000 and 3000 units of vitamin A Plasma vitamin A level was definitely higher with a minimum of about 40 μgm per 100 ml

Carotene intakes of all infants were low at birth and rose rapidly with the general caloric intake so that the final level of the older infants who were receiving several vegetables and fruit was around 200 μgm per 100 ml These infants did not show any signs of carotenemia

Each infant in this study showed a better than average rate of growth in length and weight Therefore a vitamin A intake of around 700 units daily permits normal growth and development of healthy infants We were also able to corroborate previous findings that slight illnesses result in a temporary drop in the plasma vitamin A level which may be marked However it *rises to the original level without additional vitamin A intake usually in about* two weeks

VITAMIN K CHEMICAL, PHYSIOLOGICAL, AND PATHOLOGICAL ASPECTS*

Professor DAM (Copenhagen) Vitamin K prevents hemorrhages of a type associated with defective blood coagulation due to lack of prothrombin

The simplest concept of the blood clotting process is that prothrombin a protein in the plasma in the presence of calcium is converted into the enzyme thrombin by the action of thromboplastin (also called thrombokinase) from damaged tissue or disintegrated platelets The thrombin so formed converts fibrinogen into fibrin which has the consistency of a gel

(I) Prothrombin + calcium + thromboplastin ——→ thrombin
(II) Thrombin + fibrinogen ——→ fibrin

Vitamin K acts in a yet unknown way in the formation of prothrombin in the liver

However recent investigations have shown that the first phase of the clotting process the formation of thrombin is more complicated than indicated several other factors besides prothrombin calcium and thromboplastin take part in it † The latest experiments on vitamin K deficiency in chicks

* The parenthetical numbers appearing in this discussion direct the reader to the References on page 180

† Such other factors are as follows

Owren s factors V (proaccelerin) VI (accelerin) X (proconvertin) (1 14)
Seegers plasma accelerator globulin and serum accelerator globulin (possibly identical with factors V and VI respectively) (28)
Quick s labile prothrombin factor (possibly identical with factor V and plasma globulin factor) (18)
Serum prothrombin conversion accelerator (SPCA) of Alexander et al (25)
Kappa factor of Sørbye et al (lowered in Dicumarol poisoning together with prothrombin) possibly identical with a precursor of SPCA? (22)

show that beside being necessary for the formation of prothrombin vitamin K is also necessary for the formation of one of the other factors the delta factor however in vitamin K deficiency the lack of prothrombin is of greater importance than the lack of the delta factor (23)

The two main sources of vitamin K are green vegetables and intestinal bacteria but many foods contain small amounts of vitamin K Milk is not an abundant source

Green leaves are known to contain *vitamin K* while a variant *vitamin K_2* has been isolated from putrefying protein These are the only well-defined K vitamins known to occur in nature Both compounds are fat soluble and insoluble in water They are unstable when exposed to light and oxygen and to alkali Vitamin K_1 is a yellow oil at room temperature its chemical formula is 2 methyl 3 phytyl 1 4 naphthoquinone Vitamin K_2 forms yellow crystals at room temperature (MP 54° C) its formula resembles that of vitamin K It is also a 3-substituted 2 methyl 1 4 naphthoquinone but the side chain in position 3 is longer and more unsaturated than the phytyl side chain in K Both K vitamins have been synthesized K commercially

If in the formulas of vitamins K and K_2 the long side chains are replaced by a hydrogen atom 2 methyl 1 4 naphthoquinone is obtained On an equi molecular basis vitamins K K and 2 methyl 1 4 naphthoquinone are about equally active in restoring blood coagulation in vitamin K–deficient animals but since the latter substance has a smaller molecular weight its activity per unit of weight is the highest * 2 Methyl 1 4 naphthoquinone is a yellow powder (MP 107° C) soluble in fat solvents and in hot water but very slightly soluble in water at room temperature The differences in solubilities have the following practical significance the water insoluble vitamins K_1 and K require the presence of bile salts in the intestine in order to be properly absorbed while the slight solubility of 2 methyl 1 4 naphthoquinone in water at body temperature is enough to ensure absorption even when bile salts are not present When 2 methyl 1 4 naphthoquinone is taken into the mouth a very unpleasant burning taste develops as a small amount of the substance goes into solution in the saliva

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Delta factor of Sykbye et al (lowered in vitamin K deficiency together with prothrombin) (3)
Co thromboplast n of Honorato identical with Quick s labile f ctor (8)
Prothrombokinase of M f tone (( )
Co thromboplastin of Mann and Hurn (10) probably identical with factor X (proconvertin)
Factor VII of Duckert, Loel ger and Koller (6) probably identical with fa tor X (proconvertin)

* This, at least is found when the acti it es are compared in the commonly used assay procedure with a react on period in the body of 0 hours or more

2 Methyl 1 4 naphthoquinone is produced commercially by oxidation of 2 methylnaphthalene a coal tar product *It is available as a powder or in oil solution* In the U S P 2 methyl 1 4 naphthoquinone is called *Menadione* in the B P *Menaphthone* The Scandinavian Pharmacopoeia Commission has adopted *the term Menadion for the quinone and Menadiol* for the corresponding hydroquinone which is also biologically active Menadione has sometimes been called K_3

2 Methyl 3 hydroxy 1 4 naphthoquinone *phthiocol is a yellow crystalline* substance which has been isolated from tubercle bacilli by a procedure involving treatment with alkali It is believed to have originated from a compound in this organism resembling or identical with one of the two known K-vitamins Phthiocol has some vitamin K activity but much less than menadione It is stable in light and soluble in alkaline water

A series of artificial derivatives of menadione are highly active and water soluble They are available as sterile solutions in ampuls for parenteral administration Such compounds are for instance the calcium or sodium salts of 2 methyl 1 4 naphthohydroquinone diphosphate (Synkavit or Synkayvite Roche) the sodium bisulfite compound of 2 methyl 1 4 naphthoquinone (Hykinone Abbott Laboratories) the sodium salt of 2 methyl 1 4 naphthohydroquinone-disulfate (Soluchinon Ferrosan) the hydrochloride of 4 amino 2 methyl 1 naphthol sometimes called K_5 (Kayvisyn Parke Davis & Co) the hydrochloride of 1 amino 2 methyl 4 naphthol (Synkamin Parke Davis & Co) the sodium salt of 1 acetoxy 2 methyl 4 naphthyl phosphate (Lederle)

In the body these substances are believed to be changed at least partly into 2 methyl 1 4 naphthohydroquinone and menadione When a large excess is given *some of the water soluble products may be excreted more or less* unchanged in the urine

All known forms of vitamin K natural or artificial require a certain time *from their introduction into the blood stream until* their effect in the body is fully developed None of them acts instantaneously In experimental animals with vitamin K deficiency as well as in patients with severe hypoprothrombinemia it takes several hours to restore prothrombin to about half of its normal activity and something like 18 hours to obtain the full response* In experiments with chicks the effect of a single large dose will only last for a few days but oily solutions of K_1 deposited intramuscularly may be sufficiently slowly mobilized so as to give a more protracted effect

The absorption of the fat soluble K vitamins (K and K) is supposed to

* This at least, holds for the artificial vitamin K substitutes which have been tested in relation to the reaction time

occur via the lymph external drainage of the intestinal lymph in rats leads to hypoprothrombinemia within a couple of days The condition can be corrected by parenteral administration of vitamin K (11)

Certain biological effects apart from the prothrombin formation in higher animals have been attributed to menadione and some related compounds menadione inhibits lactic acid formation in a mixture of saliva and glucose This effect is also exerted by quinones without vitamin K activity and is apparently due to a bacteriostatic action K_3' has been found to inhibit alcoholic fermentation by yeast and to act as an antibiotic against certain fungi Synkavit has been found to interfere with mitosis in tubifex eggs and has been said to retard the development of tumors caused by X ray irradiation

The clinical methods for judging the prothrombin level of the blood are mostly variations of Quick s one stage method (17) so called because it does not differentiate between the two phases of the coagulation process (thrombin formation and conversion of fibrinogen into fibrin) but tests both together In Quick s original method a large amount of undiluted thromboplastin is added together with calcium chloride to oxalated blood or plasma The time required for clotting to occur is called the *prothrombin time* because it is determined largely by the concentration of prothrombin it is however also influenced by variations in other coagulation factors which were unknown when the method was introduced The prothrombin time in the original Quick method is 11 to 11¼ seconds for blood of normal adults

Several variations of the method have been proposed in order to obtain a better differentiation between the prothrombin values in the range from 40 to 100 per cent of normal where the sensitivity of the method is unsatisfactory Such modifications are mainly of two kinds i.e that of Link and his associates who dilute the plasma to 12 5 per cent and that of Plum and Larsen who dilute the thromboplastin They also use citrated instead of oxalated plasma but this is of less importance Methods of these kinds are fairly satisfactory for checking the effect of vitamin K treatment or for use in vitamin K assay with animals although they are also influenced to a greater or lesser extent by factors other than prothrombin In contrast to this attempts to determine the amount of vitamin K itself in the blood by chemical methods have not yet been successful although procedures particularly color reactions based on more or less specific reactions of the various quinone derivatives have been described Common to all such procedures are the difficulties encountered when the task is to determine the very low concentrations which are sufficient to maintain normal prothrombin

Well-established indications for vitamin K therapy are mainly the following

The cholemic bleeding tendency
Bleeding tendency in newborn children
Bleeding tendency in celiac disease and certain cases of ulcerative colitis where the absorption of fatty material from the intestine is hindered

Excessive and uncontrolled use of paraffin oil and cathartics may be suspected of leading to unsatisfactory absorption of vitamin K

Deficiency simply due to insufficient intake of the vitamin in the diet is rare although such cases have been reported in the literature The formation of vitamin K by intestinal bacteria sets a limit to the development of hypoprothrombinemia However if simultaneously the intestinal flora is depressed by sulpha drugs or by certain antibiotics such as Aureomycin or streptomycin vitamin K deficiency is to be expected

A bleeding tendency apparently due to vitamin K deficiency has been observed in connection with hypervitaminosis A caused by eating polar bear liver which is unusually rich in this vitamin The exact etiology of this form of the disease is not yet known Perhaps depression of the formation of vitamin K by the intestinal flora is involved

A substantial dose of vitamin K reduces the prolonged prothrombin time obtained with Dicumarol and similar substances The simpler substances which in other respects may replace vitamin K_2 are almost without effect in Dicumarol poisoning It must be remembered that vitamin K and Dicumarol are not simply competitive antagonists From experiments with animals it can be seen that vitamin K deficiency leads to a lack not only of prothrombin but also of another factor in the blood coagulation system (the delta factor) *Dicumarol poisoning produces a deficiency of both prothrombin and a third* factor (the kappa factor) (5 22 23)

Administration of vitamin K along with salicylates has been advocated in order to avoid a hypoprothrombinemia supposedly caused by salicylate therapy Opinions as to the justification of this measure are divided

Vitamin K has found application in a liver function test based on the fact that in the damaged liver prothrombin formation is impaired and cannot be completely corrected by administration of vitamin K

The literature contains several statements to the effect that vitamin K menadione or certain related compounds are of value in the treatment or prevention of disorders which are not or not generally recognized as being associated with hypoprothrombinemia Convincing evidence for the justification of such therapy in these cases seems still to be lacking Examples are

Hemoptysis in pulmonary tuberculosis
Epistaxis

Bleeding from tooth extractions and other wounds of mechanical causes war casualties vitamin K has even been said to accelerate the healing of wounds
Urticaria
Chilblains
Dental caries

Reverting to the well-established uses of vitamin K one might mention the two most outstanding examples cholemic bleeding and bleeding in new born infants

1 *The cholemic bleeding tendency* is due to insufficient absorption of vitamins K_1 and K from the intestine because of the absence of bile acids It is corrected most rapidly by the intravenous administration of one of the water-soluble vitamin K active compounds say 5 or 10 mg of Synkavit The first injection must be given at a sufficient time interval before surgery is to be carried out for instance the day before and the administration must be continued after the operation until the flow of bile of normal composition with respect to bile acids is re-established which may take several weeks *The bleeding tendency will be eliminated if the condition is not complicated* by liver damage or certain rare conditions associated with hypoprothrombinemia and refractory to vitamin K For specific details of the treatment of cholemic bleeding as well as the exact use of vitamin K in the liver function test the original publications should be consulted The exact daily requirement of human adults for vitamin K is not known but it is probably *much lower than the doses used therapeutically*

2 *The bleeding tendency of the newborn* Cases of diminished clotting ability of the blood of infants were occasionally observed by Whipple in 1912 and 1913 (29 30) He found very low prothrombin in a case of melena neonatorum post mortem In 1937 Brinkhous Smith and Warner (2) *showed that the prolonged clotting time which is found in newborn infants* is due to a low prothrombin content having found that where actual bleeding occurs the prothrombin level was particularly low The first report of the fact that a low prothrombin level is common in newborn infants in the first week after birth and that it can be prevented by vitamin K therapy came from Waddell and co-workers in 1939 (26 27) Independent observations of the same kind were made by a number of other investigators among whom were Plum and his associates in Copenhagen

The result of all these investigations is that in most newborn infants the prothrombin is lower than in normal adults During the first 3 days after birth the prothrombin decreases further reaches a minimum and then rises

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Bleeding tendency in celiac disease
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Excessive and uncontrolled use of p
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tion of cases seems still to be

Hem(
Epistaxis

although the concentration of bile acid is relatively low in the bile of the newborn

Salomonsen and Nygaard (20) observed that when milk was given to a baby very early (mother's milk plus some cow's milk) the hypoprothrombinemia was less marked This observation is not in contrast to the afore mentioned conception of the cause of the hypoprothrombinemia and its relief

The order of magnitude of the minimal daily requirement of vitamin K in the first few days after birth has been determined in terms of the amount of water-soluble substitutes which must be given per day in order to secure normal prothrombin

Sells Walker and Owen (21) were the first to show that this amount is very low namely 1 to 2 μgm of Synkamin per day They inferred therefrom that even the very low content of vitamin K in milk would be enough without the aid of the intestinal flora

Larsen (9) found that 1 μgm of 2 methyl 1 4 naphthohydroquinone disuccinate (the first form of Synkavit issued by Roche) was sufficient when given intravenously and 5 μgm of it sufficed when given orally

Hardwicke (7) determined the daily minimal dose of Synkavit (the tetrasodium salt of the diphosphate) to be somewhere between 0 5 and 5 μgm when given orally

Rare cases of idiopathic hypoprothrombinemia refractory to vitamin K have been described

In the practical treatment of the hypoprothrombinemia of the newborn it is customary to give one single dose much larger than the daily minimal requirement immediately after birth the effect of which may last long enough to keep the prothrombin normal throughout the first week A dose of several milligrams of Synkavit or the like is most frequently used

Another way of keeping the infant's prothrombin time low at birth and during the critical days in the first week thereafter is to give a sufficient dose of a water soluble vitamin K substitute to the mother at proper intervals in the last weeks ante partum The necessary doses in this form of therapy and its practical value will be one of the subjects discussed by Professor Plum

It should be mentioned here that the problem of preventing or curing bleeding tendency in the newborn may go beyond the correction of the subnormal coagulation of the blood The question of capillary resistance has to be considered also Since there is no convincing evidence of an influence of vitamin K on capillary resistance other factors must be considered Under certain dietary regimens vitamin E has been found to influence capillary resistance in rat embryos and in young chicks It has also been observed that dietary

lard seemed to prevent cerebral petechiae in young rats born of vitamin K–deficient mothers Minkowski (13) has reported a beneficial influence of vitamin E on capillary resistance in the newborn

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In regard to the literature before 1948 the reader is further referred to the following two review articles

Dam H "Vitamin K its chemistry physiology and application in medicine *Advances Enzymol* 2 285–324 1942
Dam H "Vitamin K" *Vitamins & Hormones* 6 27–53 1948

REQUIREMENT OF VITAMIN K CLINICAL ASPECTS*

Professor PLUM (Copenhagen) Professor Dam has given a comprehensive review of the physiology and pathology of vitamin K and I am going to make some clinical comments

Since it is not possible to estimate the content of vitamin K in blood or body fluids we have to restrict ourselves to the estimation of the prothrombin content of the blood

Professor Dam has mentioned some of the complicated mechanisms of the coagulation of the blood which have recently been further complicated by the discovery of the new coagulation factors by Owren (12) and by Dam (3) and associates (23)

Since the estimation of the prothrombin content of the blood is essential to the discussion of the requirements of vitamin K it is perhaps appropriate to say a few words on the so-called prothrombin methods

TABLE 12

Three Principles Used in Methods for the Estimation of the Prothrombin Content of the Blood

	Dilution of plasma	Concentration of thromboplastin	Time
Warner Brinkhous and Smith (25) Thorlarson (24)	varied	constant	constant
Schønheyder (22) Dam and Glavind (1)	constant	varied	constant
Quick (18)	constant	constant	varied

According to the principle described by Quick in which dilution of plasma and the concentration of thromboplastin are constant the coagulation time has a certain correlation to the content of prothrombin

It is important that methods used for large-scale investigations be simple

*The parenthetical numbers appearing in this discussion direct the reader to the References on page 197

as well as reproduceable Therefore at the beginning of our studies we spent some time comparing the various methods of estimation finding that the Quick method was the only one that could be used on a large scale

Hjalmar Larsen and I (11) modified Quick s method so that it could be applied to capillary blood We found it essential that the blood should be mixed immediately with a large amount of citrate in order to avoid coagulation, and for that purpose we used a special pipette For the preparation of thromboplastin we used only brain and we found that the species of animal from which the brain was taken and the way in which the brain was prepared

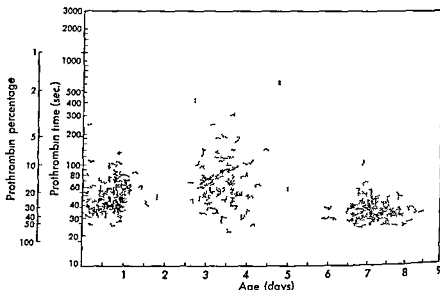


Fig 98 Three thousand determinations of prothrombin time in 1000 infants, each examined three times within the first week after birth (Plum P *Acta paediat* 38 528 1949)

were very important for the results We obtained the best results and the most suitable curve by using human brain which had not been dried

We also investigated the so-called two stage method of Warner Brinkhous and Smith (25) and the method described by Thordarsson (24) We found that a direct relationship within certain limits is found between the dilution of plasma and the coagulation time (13) We also tried to find the correlation between the prothrombin time and the prothrombin concentration (13)

The figures which follow show some of our results

Figure 98 is somewhat confusing and so I have examined the distribution of prothrombin values within 7 age classes shown in Figure 99

Figure 100 shows the distribution of prothrombin values for 180 normal

children In Figure 101 the same material is given in the way just described for Figure 99

INVESTIGATION INTO THE CAUSE OF THE PHYSIOLOGICAL HYPOPROTHROMBINEMIA IN NEWBORN CHILDREN When vitamin K is given to new born children with low prothrombin values the prothrombin content of the blood will rise to nearly the same values as found in adults When vitamin K is given to mothers before birth the prothrombin values of the newborn will be much higher than in children whose mothers have not had vitamin K This is shown in Figure 102 (10)

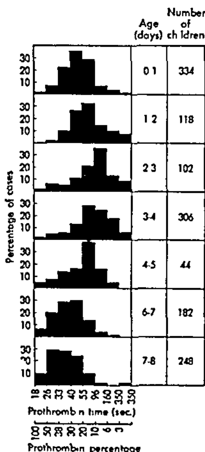


Fig 99 Distribution of prothrombin times within the first 8 days after birth The ordinate gives the percentage of children in whom were found the prothrombin times given by the abscissa (Plum, P *Acta paediat* 38 5 9 1949)

It can therefore be assumed that the physiological hypoprothrombinemia in newborn children is due to a vitamin K deficiency The cause of this physio-

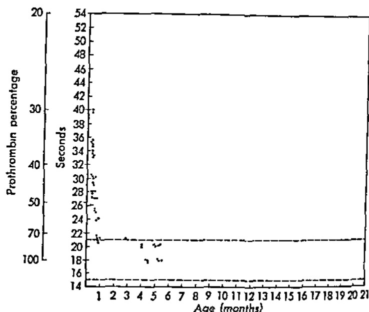


Fig 100 Prothrombin times of 180 normal children from birth to 21 months. (Plum P *Acta paediat* 38 533 1949)

logical deficiency of vitamin K is not clear The following theories have been proposed

1 Deficient supply of vitamin K from the intestine (food intestinal flora)
2 Deficient supply from the mother during the latter period of pregnancy
3 Physiological dysfunction of the liver
4 Deficient absorption of vitamin K because of decreased production of bile acids

Next I shall briefly mention some investigations carried out in collaboration with Dam Glavind (6 7) Uldall (17) Venndt (26) and Dyggve (5) related to the problems mentioned If the cause should be a deficient supply of vitamin K from the mother during the latter period of pregnancy a seasonal variation of prothrombin values of the newborn might be expected Such a seasonal variation was not found (15) as is demonstrated in Figure 103 Later we shall again return to the problem of possible seasonal factors influencing bleeding in the newborn

In order to examine the possibility of a deficient absorption of vitamin K due to a decreased production of bile acids we examined the content of bile

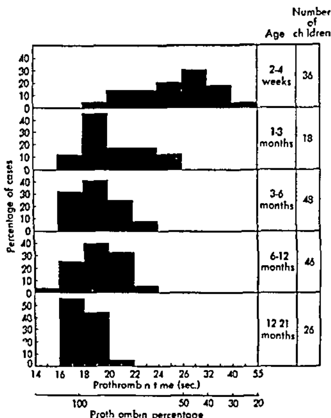


Fig 101 Distribution of prothrombin times within the first 21 months after birth The ordinate gives the percentage of children in whom were found the prothrombin times given by the abscissa (Plum P *Acta paediat.*, 38 534 1949)

acids in gallbladder obtaining the material from autopsies The results are shown in Figure 104 In newborn children we found a concentration of bile acids in the gallbladder bile somewhat lower than in older infants and definitely lower than in adults without liver disease (26)

We also questioned whether the cause of the physiological hypoprothrombinemia might originate from impaired digestion of fat and of the fat-soluble vitamin K (17) Therefore we examined quantitatively as well as qualita

tively the fat content of the feces from a number of normal infants and determined the prothrombin content of the blood during the same period Only a doubtful correlation was found with regard to the content of total fat and the proportion of split fat and unsplit fat of the feces from 11 normal breast fed infants from 1 to 7 months old

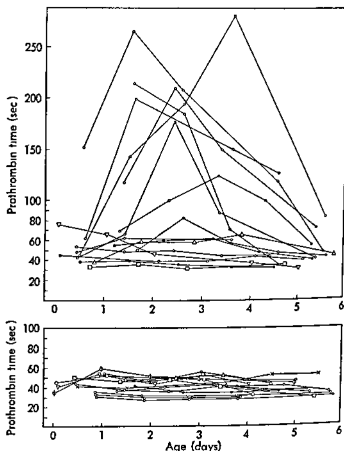


Fig 102 The upper part of the figure shows the prothrombin times of some children when the mothers had not been given vitamin K The lower part of the figure shows the prothrombin times of children the mothers of whom had been given vitamin K before birth (Larsen H and Plum P *Ugesk laeger* 102 1038 1940)

A deficient supply of vitamin K from the food or from the intestinal flora could be postulated We (6 7) examined the vitamin K content in feces from 5 normal adults and found from 60 to 230 vitamin K units per gram of dry substance (1 Dam Glavind unit is the equivalent of approximately 0 1

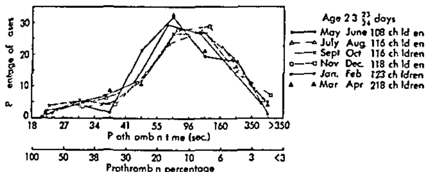


Fig 103 Distribution of prothrombin values in infants from to 4 days old examined during six consecutive two month periods (Plum P *Acta paediat* 38 531 1949)

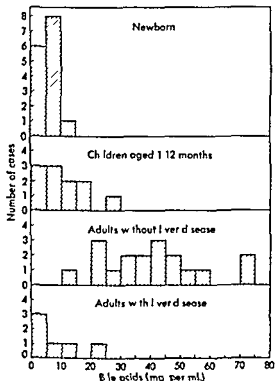


Fig 104 D tributi n of the values of bile acids in g llbladder bile in children and adults with and without l ver dise se (Venndt H and Plum, P *Acta med scand* 111 405 194)

μgm vitamin K_1) In feces from an artificially fed newborn child we found from 60 to more than 1000 vitamin K units per gram of dry substance In feces from breast fed newborn infants we found only small amounts of vitamin K from less than 5 to 50 units per gram of dry substance In feces from older children we found the same amount of vitamin K as in feces from adults In feces from normal pregnant women we found vitamin K values of the same order of magnitude as in feces from normal nonpregnant adults

The content of vitamin K in breast milk was evaluated from 29 mothers by the Dam and Glavind (2) curative chicken method at various times after delivery (7) There was found from 0 to 2 or an average 0 5 units per milliliter Four times as much was found in cow s milk in the winter season

Several authors among them Salomonsen (20) from Oslo have reported that the prothrombin content of the blood is higher in artificially fed infants than in breast fed infants We found the same difference in collaboration with Dyggve (5) A difference is seen also in the group of children whose mothers had been given vitamin K

TABLE 13

Correlation between the Diet of Infants and the Prothrombin Content of Their Blood on the Third Day (Dyggve 5)

Diet	Percentage of infants with prothrombin times more than 100 seconds		Number of infants
	Vitamin K to mothers		
	–	+	
Breast milk	57	7	1968–599
Mixed diet	27	9	462 146
Artificial diet	9	0	97–15

The difference between the prothrombin value of breast fed and artificially fed infants can to a great extent be explained by the difference in vitamin K content of human milk and cow s milk As there is a big difference in the intestinal flora of breast fed and artificially fed children and as we have found a much higher amount of vitamin K in feces of artificially fed children than in those of breast fed children the conclusion may be justified that vitamin K synthesized by the intestinal flora normally contributes part of the vitamin K needed.

In this connection I should like to mention that the stores of vitamin K can easily be depleted in infants especially during the first months of life Figures 105 and 106 will illustrate this point

Hematemesis
Vitamin A
10 mg

Prothrombin time (sec.)

Prothrombin percentage

Days

Fig 105 Boy 2 months old acute enteritis hematemesis.

200 sec.
Vitamin K
10 mg

K

Weight

Prothrombin time (sec.)

Weight (kg)

Months after admission

Fig 106 Girl 6 weeks old chronic diarrhea, at the age of 5 months, melena

In collaboration with Dam (16) I have examined the correlation between various factors at birth and the content of prothrombin of the blood of the newborn This work has been carried on by Dyggve (5) the following figures illustrating this point in part have been or will be published

From Table 14 it is seen that a positive correlation has been found between a long duration of birth and low prothrombin values of the newborn This positive correlation can be seen on the first day of life both when the mothers had been given vitamin K and when they had not received vitamin K

TABLE 14

Correlation between the Prothrombin Content of the Blood of Infants and the Duration of Birth (Dyggve 5)

Duration of birth	Age of infants	Percentage of infants with prothrombin times more than 100 seconds		Number of infants
		Vitamin K to mothers		
		−	+	
0–24 hours	1 day	11	3	233–679
24–72		17	2	67 128
72		22	14	39– 14
0–24	3 days	33	7	716–626
24–72		38	6	368 127
72		46	8	37 12

TABLE 15

Correlation between the Prothrombin of Infants and Drugs Given to Their Mothers during Labor (Dyggve 5)

Drugs given during labor	Age of infants	Percentage of infants with prothrombin times more than 100 seconds		Number of infants
		Vitamin K to mothers		
		−	+	
Barbituric acid	1 day	11	5	198 79
Chloroform		11	3	1157 365
Other drugs		13	3	1107 315
Barbituric acid	3 days	52	18	201 79
Chloroform		30	3	1138–365
Other drugs		26	9	1101–310

When barbituric acid is given to the mother during labor the proportion of infants having very low prothrombin values on the third day is larger. The administration of vitamin K to the mothers was not able to eliminate this difference (Table 15). Heavy proteinuria in the mother reduces prothrombin values in infants (Table 16).

TABLE 16
Correlation between Prothrombin Times of Infants and Proteinuria of Their Mothers (Dyggve 5)

Proteinuria	Age of infants	Percentage of infants with prothrombin times more than 100 seconds	Number of infants
No	1 day	11	1833
Slight		12	635
More than 2°/₀₀		22	111
No	3 days	34	1810
Slight		36	630
More than 2°/₀₀		40	111

TABLE 17
Correlation between Prothrombin Times of Infants and Their Weight at Birth (Dyggve 5)

Weight at birth	Percentage of infants with prothrombin times more than 100 seconds		Number of infants
	Vitamin K to infants		
	−	+	
1750 gm	11	0	38 15
1750–2450 gm	13	6	208 55
2500–2950 gm	31	15	534 65
3000 gm	33	26	1,343 217

It has often been assumed that prematures have lower prothrombin values than infants born at term. In our studies the opposite has been found (Table 17).

It will be seen that neither the administration of vitamin K to the children

TABLE 18

Correlation between Prothrombin Times of Infants and Their Loss of Weight after Birth (Dyggve 5)

Loss of weight	Percentage of infants with prothrombin times more than 100 seconds			Number of infants
	Vitamin K			
	None	Infant +K	Mother +K	
0	28	8	2	66? 90-274
50–100 gm	33	14	6	834 81 216
100–250 gm	37	25	9	691 88 176
250	43	37	18	364 68 107

TABLE 19

Correlation between Prothrombin Times of Infants and Their Condition at Birth (Dyggve 5)

Condition at birth	Age of infants	Percentage of infants with prothrombin times more than 100 second		Number of infants
		Vitamin K to infants		
		–	+	
Normal	1 day	12	19	?343–265
Asphyctic		23	9	64– 35
Other abn		13	10	172– 30
Normal	3 days	34	25	2315–?64
Asphyctic		32	0	62 33
Other abn		38	7	174 30

nor to their mothers could eliminate the positive correlation between loss of weight and frequency of low prothrombin values (Table 18)

It is seen that asphyctic children on their first day of life more often have long prothrombin times than other children This difference could be eliminated by administration of vitamin K to the babies (*Table 19*)

I think it is very difficult to account for several of the correlations demon

strated in the last six tables but I have a feeling that a discussion of the requirements of vitamin K in this period of life would be incomplete if these points were not mentioned

PROPHYLACTIC ADMINISTRATION OF VITAMIN K Since 1940 many reports have been published on the value of prophylactic administration of vitamin K to the newborn or to the mothers during the last days of pregnancy The reports vary from great enthusiasm to a completely negative attitude However many of the reports can be criticized and many of the series are not large enough

Probably the largest series has been collected by Dygge (5) from the maternity departments of the Rigshospital in Copenhagen He has gone through every case record and every autopsy over a 10 year period and besides has had the opportunity of making use of several thousand prothrombin determinations His results are shown in Table 20

TABLE 20

Frequency of Hemorrhages in 33 000 Newborn Infants (Dyggve 5)

Localization	Vitamin K before birth 10 876 infants (cases per 10 000)	No vitamin K before birth 22 371 infants (cases per 10 000)
Melena	6	16
Cephalhematoma	105	111
Intracranial	123	159
Adrenal	5	15
Subcapsular hepatic	12	18
Cutaneous	26	20
Umbilical	1	9

The frequency of melena was reduced to about one third and the frequency of umbilical bleedings was even further reduced The frequency of intracranial hemorrhages was somewhat reduced and to our surprise the adrenal hemorrhages were much lower in the vitamin K–treated group I think this last finding needs further confirmation

The reduction of intracranial hemorrhage is seen both in infants born at term and in prematures We have tried several ways of administration and several preparations of vitamin K and we think it is important to bear in mind that the effect of prophylactic administration of vitamin K is very much dependent on these factors Table 21 is a comparison of the effect of two various synthetic vitamin K preparations and it is seen that one of them the

TABLE 18

Correlation between Prothrombin Times of Infants and Their Loss of Weight after Birth (Dyggve 5)

Loss of weight	Percentage of infants with prothrombin times more than 100 seconds			Number of infants
	Vitamin K			
	None	Infant +K	Mother +K	
0	28	8	2	667 90 274
50–100 gm	33	14	6	834 91 216
100–250 gm	37	25	9	691 89 176
250	43	37	18	364 68 107

TABLE 19

Correlation between Prothrombin Times of Infants and Their Condition at Birth (Dyggve 5)

Condition at birth	Age of infants	Percentage of infants with prothrombin times more than 100 seconds		Number of infants
		Vitamin K to infants		
		−	+	
Normal	1 day	12	19	2343–765
Asphyctic		23	9	64–35
Other abn		13	10	172–30
Normal	3 days	34	25	2315 764
Asphyctic		32	0	62 33
Other abn		38	7	174 30

nor to their mothers could eliminate the positive correlation between loss of weight and frequency of low prothrombin values (Table 18)

It is seen that asphyctic children on their first day of life more often have long prothrombin times than other children This difference could be eliminated by administration of vitamin K to the babies (Table 19)

I think it is very difficult to account for several of the correlations demon

strated in the last six tables but I have a feeling that a discussion of the requirements of vitamin K in this period of life would be incomplete if these points were not mentioned

PROPHYLACTIC ADMINISTRATION OF VITAMIN K Since 1940 many reports have been published on the value of prophylactic administration of vitamin K to the newborn or to the mothers during the last days of pregnancy The reports vary from great enthusiasm to a completely negative attitude However many of the reports can be criticized and many of the series are not large enough

*Probably the largest series has been collected by Dyggve (5) from the ma*ternity departments of the Rigshospital in Copenhagen He has gone through every case record and every autopsy over a 10 year period and besides has had the opportunity of making use of several thousand prothrombin determinations His results are shown in Table 20

TABLE 20

Frequency of Hemorrhages in 33 000 Newborn Infants (Dyggve 5)

Localization	Vitamin K before birth 10 876 infants (cases per 10 000)	No vitamin K before birth 22 371 infants (cases per 10 000)
Melena	6	16
Cephalhematoma	105	111
Intracranial	123	159
Adrenal	5	15
Subcapsular hepatic	12	18
Cutaneous	26	20
Umbilical	1	9

The frequency of melena was reduced to about one third and the frequency of umbilical bleedings was even further reduced The frequency of intracranial hemorrhages was somewhat reduced and to our surprise the adrenal hemorrhages were much lower in the vitamin K–treated group I think this last finding needs further confirmation

The reduction of intracranial hemorrhage is seen both in infants born at term and in prematures We have tried several ways of administration and several preparations of vitamin K and we think it is important to bear in mind that the effect of prophylactic administration of vitamin K is very much dependent on these factors Table 21 is a comparison of the effect of two various synthetic vitamin K preparations and it is seen that one of them the

TABLE 21
Effect of Two Synthetic Vitamin K Preparations on the Prothrombin Times of Infants*

Prothrombin times (seconds)	Hours before delivery 23–4 (20 mg) disulphate		3¾–1 (20 mg) disulphate	
	(20 mg) disulphate	(40 mg) disuccinate	(20 mg) disulphate	(40 mg) disuccinate
30	3	15	2	14
30–50	59	76	41	66
51–75	21	8	24	15
76–150	3	0	17	5
150	15	2	15	1
	Percentage of infants			

* Comparison of the effect on prothrombin times of infants of the prophylactic administration by mouth to their mothers of two synthetic vitamin K preparations (5) The preparations were the sodium disulphate ester and the sodium disuccinate ester of 2 methyl 1 4 naphthohydroquinone

disulphate ester has much less effect than the other one the disuccinate ester The hemorrhages occurring as a result of coagulation defect such as are seen in hemophilia in fibrinopenia and in hypoprothrombinemia are due to two factors trauma and the coagulation defect Therefore it is natural and well known that a person or an experimental animal may have a considerable degree of coagulation defect and still have no bleeding We have questioned whether there was any difference in the degree of hypoprothrombinemia between children who had hemorrhages and children who had not Dyggve found that pronounced hypoprothrombinemia was seen more often in children who had hemorrhages than in children without hemorrhages In order to exemplify the difficulty involved in this problem I should like to mention a recent report in the *American Journal of Diseases of Children* 1949 by Sandford Kostalik and Blackmore Their conclusion reads Our studies indicate therefore that the administration of vitamin K to premature infants in an endeavor to decrease haemorrhagic manifestations is without clinical basis This is very clear But when one analyzes the report the conclusion does not seem warranted The study comprised 100 premature infants Eighty three were not given vitamin K 17 were given vitamin K The 83 infants not receiving vitamin K were all born in the hospital and cared for in the nursery for premature infants However the children in the treated group beside being a very small group of 17 were delivered in other hos

pitals and only 12 of them were brought to the nursery The authors state *Those infants given vitamin K were born outside our hospital under poor conditions of delivery and care* —and further— *For years the mortality of premature infants born outside this hospital has been three to four times greater than that of those born within the hospital* In the untreated group there was a total of five infants who showed hemorrhagic manifestations two exhibited melena one hematemesis one umbilical hemorrhage and one ecchymoses none had signs of cerebral hemorrhage All of them survived without treatment In the group of 17 infants given vitamin K there were four infants with hemorrhagic manifestations But the information given in the report about these four is incomplete one had melena and hematemesis and died one day after birth In the prothrombin test a clot could not be obtained but no effort was made to find the reason for this Permission for autopsy was not obtained It is not stated whether the melena was of such a degree that it might have been a cause of death Children with severe cerebral hemorrhage often have hematemesis But the important point is that we do not know why this infant died One infant had some hematemesis on the second day No further statement is given This hematemesis may have been very slight we do not know Two infants died of cerebral hemorrhage it is said in the report but a few lines further it is stated in both these infants there were asphyxial disturbances that were probably more a cause of death than the cerebral hemorrhage" So we see that the two groups treated and not treated cannot be compared since one group was very well cared for while the other group had poor conditions of delivery and care Next we see that we know too little of the vitamin K-treated infants with hemorrhages The time of administration of vitamin K is only stated in two cases the kind of preparation is not mentioned and it is not stated whether the mothers had vitamin K or not When the authors further put in a table giving the total mortality in both groups irrespective of hemorrhages they arrive at the conclusion that the mortality in the untreated group was 12 per cent and in the treated group 47 per cent and as they seem to have forgotten the infant without autopsy the mortality was really over 50 per cent However the authors wish to emphasize that the great differences in mortality between the two groups is in no way due to the administration of vitamin K"

SEASONAL VARIATION OF HEMORRHAGES IN THE NEWBORN Many authors have found a seasonal variation of melena in the newborn Salomonsen (19) stressed the seasonal variation in reports from Norway in 1939 Kepila and Leppo (8) from Finland in 1937 Wirz (28) from Switzerland in 1941

Kerpel Fronius (9) from Hungary in 1944 and Winkler (27) from Germany in 1938 reported similar findings Also in Canada and Japan the same seasonal variation has been found Dyggve s figures are given in Table 22

TABLE 22
Influence of the Season on the Frequency of Intestinal Hemorrhages (Dyggve 5)

	Percentage	Number of infants
Winter	20	7
Spring	40	14
Summer	23	8
Autumn	17	6

The same seasonal variation has been found in the frequency of intracranial hemorrhages by Kerpel Fronius and by Dyggve Dyggve s figures are given in Table 23

TABLE 23
Correlation between Season and Frequency of Intracranial Hemorrhages in Premature Infants Number of Cases per 10 000 Birds (Dyggve 5)

	Vitamin K to mothers	
	–	+
Winter	78	57
Spring	88	56
Summer	57	40
Autumn	27	26

As can be seen the number of intracranial hemorrhages is much lower in summer and still lower in autumn than in winter and spring The difference is not eliminated in the group of children who received prophylactic vitamin K through their mothers As a similar seasonal variation of the prothrombin values could not be found both Dyggve and Kerpel Fronius draw the conclusion that a factor other than vitamin K may be of importance Kerpel Fronius has suggested that vitamin P could be the factor in question but considering the somewhat doubtful nature of this vitamin I think we have to think of other possibilities for instance vitamin E or some other still unknown factor

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VITAMIN B COMPLEX IN INFANTS

Dr CLEMENTS (Geneva) I am going to limit myself to thiamin along with a few words about riboflavin What I am going to say is taken from studies made by a team in Australia before I joined the World Health Organization

The level of thiamin in colostrum is quite low about 1 or 2 μgm per 100 ml The level in milk rises to about 10 μgm in about 10 days and reaches a plateau of about 20 μgm in a month It is possible to raise the thiamin content of maternal milk to about 30 μgm if very large doses of thiamin are given to the mother but that seems to be the ceiling

It became obvious during the study that infants exhibited signs of deficiency if the maternal milk contained less than 12 to 15 µgm of thiamin per 100 ml for any considerable period. These signs are quite common in infancy and for a while we were skeptical that they could be associated with partial thiamin deficiency. The signs are failure to grow in weight at the normal rate, vomiting which may become projectile, and constipation. The diagnosis is confirmed in two ways: by finding the thiamin content of the maternal milk to be low and by the effect of treatment on the child. In our series we found that thiamin administered to the child, without any dietary change, corrected the situation.

In one case the mother began dieting of her own volition. She completely changed her dietary pattern so that her thiamin intake was reduced to about a third of what it had been. Fortunately for this particular study we had a long series of thiamin assays on her milk. Just before dieting began it was in the vicinity of 22 µgm per 100 ml. A fortnight before the infant was diagnosed as *being thiamin deficient her milk contained 5 to 6* µgm per 100 ml. Thiamin was administered to the infant, with a resultant gain in weight, cessation of vomiting, and disappearance of constipation.

The time of onset of these symptoms is between the twelfth and sixteenth week. The earlier cases were observed in infants whose mothers' dietary regimen during pregnancy had been quite poor. The later cases occurred in the infants of mothers whose diet apparently had been satisfactory. The method is somewhat unsatisfactory because it required assessing diets in retrospect, which method is always suspect.

From this study we can obtain some idea of the thiamin requirements during infancy. We may assume that the low thiamin content of breast milk during the first month is compensated by storage in the fetus. Therefore we can neglect the thiamin content of milk for the first month and look at levels after that period. During this time the infant is growing very rapidly. The ratio of thiamin to nonfat calories in human milk at this time is about 0.63. The thiamin intake of well nourished, healthy, breast fed infants from 2 to 4 months of age, whose mothers have been on a good diet, is about 200 to 250 µgm daily. We have used the thiamin content and the thiamin nonfat calorie *ratio of human milk (0.63) to measure thiamin requirements of infancy.*

The appearance of cases of partial thiamin deficiency indicates that there is not a big margin between the actual supply and the requirements, for when the intake, over a period of time, drops to half the maximum, it seems that the infant is likely to develop clinical deficiency.

It is interesting to speculate that some of those infants who are weaned because the mother's milk does not agree with them may actually present cases

of partial thiamin deficiency For if an infant is weaned onto cow s milk mixture which consists of 50 per cent or more of cow s milk then it is go ng to receive considerably more thiamin because the thiamin content of cow s milk is at least twice that of human milk Obviously not all infants who are weaned from mother s milk suffer from partial thiamin deficiency Some in fants undoubtedly must be put on cow s milk because of the high fat content in human milk

One observation made in this study was that any disturbance in the health of the mother such as a low grade chronic infection just sufficient to make her uncomfortable and give her a slight temperature would reduce the thiamin content of the milk to half its usual value In some of our patients the origin of the deficiency in the milk could be ascribed to such mild infections

We began to study riboflavin but unfortunately did not complete the study As far as we went however we were unable to detect any signs or symptoms in breast fed infants which could be attributed to an inadequate intake of riboflavin Furthermore the range of fluctuation in riboflavin content of human milk was not large and none of the mothers had low levels In a few mothers however an infection reduced the riboflavin content of the milk although the degree of reduction did not seem to be as great nor to last as long as it did in thiamin deficiency

Professor LEHMANN (Goteborg) I shall present the results of an investigation on the use of vitamin K as a prophylactic agent against bleeding in infants The experiments were conducted at the women s clinic Sahlgren s Hospital Goteborg Sweden

The study was begun 1 September 1940 and is still continuing At the end of this year about 50 000 infants will have been treated The figures I am presenting here concern the 13 250 infants treated during 1940–1943

Before starting the treatment the neonatal physiological hypoprothrombinemia was measured in 40 infants in July and August when plenty of vegetables were available and in 50 infants in March and April when we were rather short of vegetables The prothrombin index (PTI) was determined by a micromodification of Quick s one stage method (*Monatsschrift fur Kinderheilkunde* 86 44 1941 and *Svenska Lakartidningen* 39 2253 1942)

The hypoprothrombinemia was more severe in March and April than in July and August In the latter series 7 5 per cent of the infants had an index below 20 (corresponding to 5 per cent prothrombin) and 30 per cent had an index below 30 The corresponding values for the March and April series were 42 per cent and 76 per cent respectively

The dosage of vitamin K for prophylactic treatment was 1 mg of sodium

2 methyl 1 4 naphthohydroquinone disulphate given orally This amount was chosen since it was found to have the same effect as 5 or 10 mg even if the PTI was below 10 (= 1 per cent of the normal prothrombin content) It was of particular interest to see how quickly the prothrombin level rises after this dose The PTI rises from 5 to over 20 in 6 to 8 hours and is the same for both oral and intramuscular administration Above a PTI of 20 we seldom see bleeding in infants from blood sample needle punctures Naturally there is a risk of bleeding during these 6 to 8 hours However the PTI values just after birth usually lie between 30 and 60 and the vitamin K thus produces a rise from these levels and not from very low values

The results of the prophylactic treatment are found in the number of deaths caused by bleeding among 13 250 full term infants As control material we have 17 740 untreated infants born between 1934 and August 1940 The death rate in the control group was 1 94 per thousand as compared with 0 45 per thousand in the treated group The difference is statistically significant and shows that 1 5 per thousand full term infants can be saved by treatment with vitamin K Thus in Sweden with about 100 000 full term infants born each year 150 infants may be saved yearly by prophylactic treatment

When we compared deaths on different days after birth it was evident that the greatest reduction in deaths during the period when vitamin K was given, occurred after the first 24 hours indicating that the deaths during the first day were chiefly due to trauma which led to lethal hemorrhages

As to the localization of bleeding it was found that there was a reduction in deaths from all forms of bleeding but especially in deaths from intracranial hemorrhage indicating that the latter should be included under hemorrhagic diseases of the newborn

The treatment of infants after birth with vitamin K is easy and cheap but it is not the most effective form of prophylactic treatment Treatment of the mother before delivery is preferable as the infant is then born with normal PTI values between 60 and 90 We have tried different *regimens for* medication single doses just before delivery and small daily doses during the last two weeks before delivery For the vitamin to be effective in the infant it must be given to the mother at least 6 to 8 hours before delivery This time limit depends somewhat on the initial level of the fetus but in general 6 to 8 hours can be considered a safe minimum before delivery As many mothers deliver within 1 to 6 hours after arrival at the hospital the obvious conclusion is that the medication must be given before their arrival at the hospital

Professor SALOMONSEN (Oslo) Professor Plum has compared the influence of artificial vs breast feeding on the prothrombin time of the new born infant I think Professor Plum has misunderstood my investigations

The point is not in a comparison of feeding with bovine or human milk but in a comparison of *early* feeding during the first day of life with feeding started later during the second or third day. In my material every child received human milk but one group had an additional small amount of cow's milk first given two hours after birth. In that group the prothrombin time and the coagulation time were much lower than in the group which had only human milk. I think Professor Plum demonstrated that there is a correlation between prolongation of the prothrombin time and loss of weight. I found the same result and I think it may confirm the finding that early feeding has an influence upon prothrombin time. Early feeding will also prevent much loss of weight.

Professor Plum has found that there is a seasonal variation in the occurrence of hemorrhagic disease. I found quite the same. Of 66 cases I observed 44 occurred in the winter and spring and only 22 cases occurred in summer and fall.

From my experience I would say there is also a seasonal influence upon the physiological prolongation of the prothrombin and coagulation times in the newborn. I want to stress it because I think it shows that some exogenous factor is influencing this physiological disturbance, perhaps the food of the mother. In Norway the mothers get very few vegetables during late winter and spring. Perhaps conditions are different in Denmark and that is the explanation of the difference in our findings.

CHAIRMAN: This question about early feedings is, I suppose, connected with the modern views on late feeding in premature babies which recommend that feeding be started on the second, third, or fourth day of life. In addition to the starving effect on vitamin K production, premature babies are especially apt to get hypoprothrombinemia.

Professor PLUM (Copenhagen): I am sorry if I did not mention Professor Salomonsen's work correctly but I have certainly not misunderstood him. We have worked along the same lines. Dr. Hjalmar Larsen has carried out some interesting work which I did not report in detail, partly because I do not know enough about it but also because it is not yet finished. He has tried to give as much human milk as possible to the infant immediately after birth. He found that he could not prevent loss of weight in every case; therefore there must be a factor other than starvation producing weight loss in some cases. Nevertheless, the effect of immediate feeding after birth, whether of human or bovine milk, was very salutary. Based on the amount of vitamin K in human milk, it should be possible to prevent a deficiency of vitamin K with milk alone if the child receives enough. We found 0.5 units of vitamin K per milliliter, which is equal to 1 μgm. of vitamin K per 10 to 20 ml. Ac-

cording to the requirements mentioned by Professor Dam it should be easy to give enough human milk to supply sufficient vitamin K But the problem is complicated by the vitamin K content of the fetus and by the unknown extent to which vitamin K is absorbed from the intestine Although rather complicated I agree that if we feed the newborn early, a gratifying effect on low prothrombin levels will result

With regard to the seasonal variation there seems to be a geographical difference Such variation seems to be found in Norway and Sweden but not, as Figure 103 (p 187) shows in Denmark

CHAIRMAN We have not heard any explanation of this seasonal variation that is found in some countries at least Is there anybody who can tell us something about it?

Professor YLPPÖ (Helsinki) We have also seen seasonal variation in Finland in the occurrence of hemorrhages among the newborn But the mortality in tuberculosis pneumonia and other infectious diseases increases at this time as does total mortality So this is a general phenomenon If we consider melena as a typical disease with severe hemorrhage we often find that mothers of these children have had pneumonia or influenza so that there is clearly an infectious element present With other hemorrhages such as in the brain of the newborn no seasonal variation can be noted There must be other factors such as compression of the skull stasis in the lungs dilatation and rupture of capillaries and other mechanical causes Of course it is quite clear that vitamin K also plays a role but we must not forget other possible explanations for hemorrhages

Professor LEHMANN (Goteborg) I was born in Denmark and have lived half my life there and half in Sweden When I moved to Sweden I was surprised to find that the Swedes did not eat as many vegetables as the Danes This is changing but there is still a lack of vegetables here compared with Denmark which may provide an explanation for the seasonal differences I do not know about the vegetable intake in Switzerland but Willy in Zurich has not found any seasonal variation in hypoprothrombinemia

CHAIRMAN Dr Clements has spent most of his time in Australia and a part of his time in Switzerland

Dr CLEMENTS (Geneva) As you all probably know the Food and Agriculture Organization of the United Nations asks each of its member countries to furnish food balance sheets of production and consumption I had occasion to study these balances for the years 1947 and 1948 just before I came here and I can confirm Professor Lehmann s observation and add figures on Switzerland In Switzerland if you set the consumption per capita of

green leafy vegetables at 100 the consumption in Denmark would be about 60

CHAIRMAN The carotene content of the blood in the different countries would be of interest to know

Professor BESSEY (Chicago) There is one point that Professor Dam raised about capillary resistance on which I would like to comment I think it is not generally realized how important prompt clotting time is on the apparent resistance of capillaries If one produces very mild trauma to the skin of animals such as drawing a pencil across the area and then examines that area by making microscopic sections he will find numerous breaks in the capillaries My point is that capillaries are very very fragile and although a small trauma causes breakage it never leads to hemorrhage because clotting is so rapid If there is something wrong with the clotting mechanism then such mild abrasions lead to what one can recognize as very small hemorrhages

Professor STEARNS (Iowa City) We have been studying in collaboration with our departments of obstetrics and ophthalmology the relationship of vitamin K given to the mother before birth to the incidence of visible retinal hemorrhage The late Dr Kirsten Toverud started the study because in Norway they had found evidence of small resolved capillary hemorrhages in infants born dead In New York it had been found that 40 per cent of the infants born to indigent mothers showed evidence of small retinal hemorrhages which usually disappeared within one or two weeks In our preliminary control series of about 250 mothers we found an incidence of only 25 per cent In those mothers who received 10 mg of vitamin K for a period of 2 days to 2 weeks before delivery the incidence dropped to 15 per cent This correlation was better than that between the incidence of these small hemorrhages and the one stage Quick prothrombin method although there was correlation there We felt that the results justified further study

Professor PLUM (Copenhagen) Dr Willy found retinal hemorrhages in 30 per cent of all newborn children with a somewhat lower incidence in the group treated with vitamin K There was no evident correlation between the level of prothrombin in the blood and the appearance of retinal hemorrhages

Professor STEARNS (Iowa City) In Dr Smith s review in a symposium on vitamins he has a chart with prothrombin times from various investigators checked against the dietary history of the mothers I think he felt very strongly that the prothrombin time of the infant during the first three days of life was correlated to the diet of the mother in regard to vegetables

Professor DAM (Copenhagen) What was the symposium?

Professor STEARNS (Iowa City) It was in the published symposium

which was edited by E A Evans Jr of Chicago the title of the volume was *The Biological Action of the Vitamins* (The University of Chicago Press, 1944)

Professor LEVINE (New York) Dr Potter believed that there was no relation between the incidence of hemorrhagic disease in the newborn and the administration of vitamin K

Dr CLEMENTS (Geneva) It seemed to me that Professor Plum gave very high figures for vitamin K concentration in feces Is there any information about the levels at which vitamin K is absorbed and as a corollary to that is the vitamin K imprisoned in feces available to the body?

Professor DAM (Copenhagen) I think it is generally believed that the intestinal tract furnishes vitamin K but as to the level or the amount needed I do not know In 1939 and 1940 the late Professor Orla Jensen and I determined the vitamin K in some intestinal bacteria Some strains of B coli had about 1000 units per gram of dry weight

When the bacteria are grown under different conditions the content of vitamin K varies A few months ago we tried to repeat these determinations with some other strains of colon bacillus from Orla Jensen s culture collection and discovered only 1/10 or 1/20 the amount found in the strains which he grew in 1940

One more thing could be said about the synthesis of vitamin K by bacteria the vitamin K is in the cell of the bacteria and not in the liquid media in which it grows Therefore any vitamin K supplied by the intestinal bacteria must be the result of the death and dissolution of the bacteria As long as they are alive the vitamin K cannot be transferred to the body

Professor LEVINE (New York) I should like to ask what is the consensus of the experts here should premature as well as full term infants be given vitamin K by mouth in preference to parenteral administration?

Professor LEHMANN (Goteborg) We found that we were not sure that premature infants swallowed what we gave them and so all premature infants in Goteborg are given 5 mg synthetic vitamin K by injection

Professor WALLGREN (Stockholm) But even if you give every premature infant vitamin K in the delivery room these children will still have a lower prothrombin index than full term infants Even though prematures receive vitamin K at birth they may have a prothrombin index sufficiently low to produce bleeding

I wish to raise the question of constitutional hypoprothrombinemia What is the cause of that? Is it due to a lack of vitamin K absorption or production or is it due to disturbance of liver function?

Professor PLUM (Copenhagen) *It is well known that patients with*

heart disease often have a lower prothrombin level than normal and that this level is not elevated by vitamin K given either by injection or by mouth I have seen two or three cases with idiopathic hypoprothrombinemia which could not be elevated by any vitamin K

Professor WALLGREN (Stockholm) There was no liver damage evident?

Professor PLUM (Copenhagen) Not enough to be demonstrable

Professor RAIHA (Helsinki) Suppose there is a change in the blood flow to the liver as in heart disease and as perhaps in prematures because of an open ductus venosus arantii Could that be the reason for the lower content of prothrombin?

Professor DAM (Copenhagen) The hepatic blood flow might influence the output of prothrombin from the liver

Professor LEVINE (New York) After a dose of 6667 U S P units of vitamin A the rise in the plasma vitamin A of a group of premature infants was just about half the rise in the full term group This indicates either in effective absorption of vitamin A by the prematures or a depleted vitamin A storage in the body of the premature infant

In the livers of infants who died 24 hours after birth the vitamin A per gram of liver was more than twice as high in the full term group as in the premature group and in the total liver the content of vitamin A in the full term group was five times that of the prematures I should like to ask Professor Stearns whether she has any idea of the optimal dose of vitamin A for premature infants on the basis of these data

Professor STEARNS (Iowa City) I am not certain but I think that 700 units is too low It would not be enough for storage and it would probably not provide enough in case of illness or abnormal destruction of A But I would not venture to guess the optimal dose for either group

Professor BESSEY (Chicago) There are several ways of getting at this matter of requirement and I want to mention one other way that is a little different If you place an infant on an amount of thiamin decreasing grad ually each day you reach a point where there is a minimum excretion in the urine If you drop the intake still further you still maintain that excretion This has been used by Holt and others as a unique point of intake believed to have some significance with respect to requirement If the intake drops below this level the concentration in the tissue is decreased in experimental animals of several species which it has been possible to study It may well be that where intake is such that you do not maintain tissue at maximum con centration the intake may be below the requirement Thus if you gave a mother a thiamin intake that was below the minimum excretion point of

thiamin for that mother she would produce breast milk that was below the optimum level of thiamin In other words it would drop below 200 μgm per cent and as Dr Clements pointed out 200 μgm per cent seems to cover the infants requirements The margin is narrow and if it drops very much from that you do observe symptoms of thiamin deficiency in the infant I merely want to point out first that this is another way of looking at this very difficult problem of determining minimum requirements and second that thiamin at its highest concentration in mother s milk does not give very much of a margin

Professor LEVINE (New York) Using the same type of reasoning what is the situation with respect to riboflavin?

Professor BESSEY (Chicago) Measuring requirements for both thiamin and riboflavin by this technique indicates that the infant s need for these two materials is quite out of proportion to the body weight as compared to the adult requirement If you multiply the requirement of the infant on a kilogram basis by 70 you get a figure that we know is two or three times the requirement of an adult This may indicate that the infant requirements may be quite a lot more on a per kilogram basis than those for an adult

Professor PLUM (Copenhagen) In 1945 Potter published material consisting of about 6000 children who received vitamin K and about the same number who did not She found no difference in the mortality The criticisms that could be raised are that first the dose of vitamin K was very small only 3 mg given to the mothers by intramuscular injection second more than half of the mothers had the injection a few hours before birth third only bleeding in dead children was recorded and the frequency of such bleeding was surprisingly low For instance she found only 7 cases of cerebral hemorrhage among the dead children and only 14 among the living children She found no hemorrhagic disease of the newborn at all among the dead children and only two cases in the living children She did not take into consideration whether the children received vitamin K or not

Professor DAM (Copenhagen) I just want to recall the results of the Hungarian pediatrician F Gerloczy who has recorded some studies in scleredema in the newborn He claims that vitamin E has a striking effect in preventing or curing this disease My interest in this is that Gerloczy referred to some animal experiments carried out in my laboratory My associates and I had found in young chicks a condition which appears to resemble scleredema in infants I have three publications by Gerloczy The first appeared in the Hungarian journal *Pediatria Danubiana* (2 No 5 1947) and is entitled Vitamin E Treatment of Scleredema in Premature Infants Then in 1949 he published a brief review of his studies in the Swiss journal

Experientia (5 252 1949) and a more detailed study in the same year in *Annales paediatrici* (173 171–86 1949) I shall begin by reading his summary of this article and then tell you something about the animal experiments which he refers to I am not acting as an advocate of Gerlóczy s opinions since I am not able to decide whether his claims are correct Maybe you will be able to judge

1 Classification differentiation pathology and etiology of scleroma are discussed The author states that the disease is more frequent with premature babies weighing over 1500 gm The number of cases is on the increase Premature babies who develop scleroedema have a much worse prognosis than premature ones without scleroedema

2 There is no reference in the literature to vitamin E therapy in scleroedema

3 The investigations of the author were induced by the clinical observation of catastrophal diuresis and loss of weight in an infant treated for scleroedema with vitamin E

4 The results of earlier investigations are summarized Vitamin E invariably produced severe diuresis When given intramuscularly it had no effect A cautious dosage was used to prevent loss of body weight for two days 5 mg daily were given per os If the result was satisfactory i e if no diuresis or loss of weight occurred the same dosage was continued for not more than 5 days Urinary output and body weight were carefully observed The results were good

5 The later prognosis of the infants treated with vitamin E is favorable

6 The poor prognosis is due to the oedema of the vitally important internal organs Scleroedema is merely a partial phenomenon of general oedema The presence of scleroedema is indicative of visceral oedema Vitamin E exerts a draining action on visceral oedema and scleroedema alike and it is by this effect that the prognosis is improved This assumption has received much support by animal experiments and by a few corroborating data from human pathology

7 Scleroedema mainly occurs on the 2nd or 3rd day of life At this age the new born is very sensitive to vitamin E diuresis occurs The later we administer vitamin E the less effect it has in draining the patient Vitamin E has no effect in mature infants and premature ones without scleroedema

8 Great precaution has to be taken in administering vitamin E to infants with scleroedema No other sort of fluid accumulation can be mobilised by vitamin E enormously high as its dose appears to be Apart from the scleroedema the dancer from overdosage is nil

9 Scleroedema can after the onset of milk secretion be cured by giving vitamin E to the mother only

10 None of the infants who were *given 5 mg of tocopherol per day prophylactically* from the first day of life developed scleroedema *

Gerlóczy studied the occurrence of scleredema during the prewar war and postwar years in two departments of obstetrics in Budapest Each year from

* *Ann paediat* 173 183–84 1949

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were fed That was with another purpose in mind following the report that retrolental fibroplasia could be prevented by the use of vitamin E In those normal premature babies we did not observe a diuresis One thing that might be related to your animal observations is that in two of the 49 babies a cataract developed We have never seen a cataract in premature babies of acquired and postnatal origin When cataracts of the nuclear type occur they are usually congenital and discoverable at birth In these two babies the eyes were entirely normal until about 3 or 4 months after birth Now I do not know whether a drying effect on the lens could be related to the drying effect on edema That is pure speculation

Professor DAM (Copenhagen) Is anybody aware of cerebral changes other than the cerebral hemorrhages which Dr Minkowski has described? In chicks very marked cerebral or cerebellar symptoms can develop on a vitamin E-free diet of particular composition The animals become ataxic have convulsions and behave quite crazily Sometimes the changes in the brain can only be seen microscopically but often it can be seen macroscopically just by opening the skull These changes were first described by Pappenheimer and Goettsch and called encephalomalacia Gerlóczy did not observe symptoms of this kind They are not seen in rats either I have never heard of anything like this in human prematures

to our hospital many instances of infection were occurring in the big maternity hospital where these infants had been born So I think the increase in scleredema neonatorum is due to infection

Dr ZETTERSTRÖM (Stockholm) Can it be that the pathological changes observed in vitamin E deficiency are due to damage to the small blood vessels and that the necrosis and edema are secondary changes? I should think that the improvement in the condition after administration of vitamin E could also be due to an effect on the blood vessels

Professor DAM (Copenhagen) Yes I think there is an effect on the capillaries and the resistance of the capillaries In the chick at least when the symptom is precipitated by easily autoxidizable fat it is likely that the oxidation which the fat undergoes in the tissue in some way damages the capillaries But there also seems to be less resistance of the capillaries if no fat is given, because then we meet the other extreme lack of essential fatty acids

Professor RÄIHÄ (Helsinki) I believe I have seen a paper from Dr Minkowski about capillary resistance

Professor BARNETT (New York) At our discussion in Leyden Dr Minkowski reported that he did not believe they had observed any increase in the incidence of this disease in Paris The incidence there is very low and in the very few babies they had treated they had also observed a diuresis and rapid subsidence of the symptoms The cases were too few for him to say anything about them other than that they tended to confirm the observation that vitamin E did affect this disease

I am not certain how much edema there was other than that of the skin in the patients he had treated

Professor LEVINE (New York) He said his aim was to reduce intracranial hemorrhage in premature babies and so he did not use it principally for scleredema in premature infants but because of its effect in increasing capillary resistance He measured the fragility of the capillaries by a suction method and found that the fragility varied inversely with the weight of the premature He tried vitamin E vitamin C vitamin P and some of the quinones and he agreed with the previous reports of the antihemorrhagic properties of vitamin E He gave mothers of premature babies 600 to 900 mg of *dl*-α tocopherol acetate during labor When they expected a premature baby he gave the mother vitamin E just prior to delivery He used 105 tests and 105 controls in different weight groups The vascular resistance of the offspring was increased in each group of test babies over the controls

We have given vitamin E to about 50 premature babies the first time they

absorbed by soda lime in the spirometer O_2 consumption is considered to be equal to the total decrease of gas volume in the system This decrease is recorded on a kymograph and the pump is stopped for a few seconds after every fifth minute so that we can check the constancy of O_2 consumption by

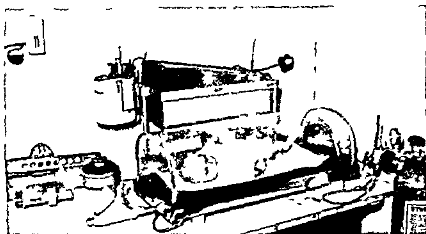


Fig 107 The apparatus used for basal metabolism determination in infants

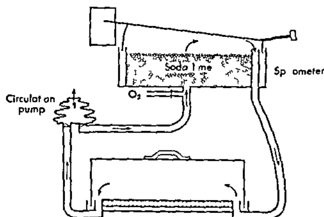


Fig 108 Schemati diagram of apparatus of Figure 107

comparing the tracings from these five minute periods with one another A typical curve from a six month-old child is shown in Figure 109

If the decrease in gas volume is to be considered equal to the O_2 consumption of the child the CO heat and moisture uptake in the system must eq

CHAPTER V

Panel on Basal Metabolism

METHODS OF DETERMINING BASAL METABOLISM IN RELATION TO BODY MEASUREMENTS, BLOOD VOLUME, AND HEART VOLUME

Dr KARLBERG (Stockholm) Basal metabolism can be measured in two ways by direct calorimetry in which production of heat per unit time is measured and by indirect calorimetry in which this heat production is calculated from measurements of O_2 consumption and/or CO_2 output per unit time Direct methods are difficult and are not commonly used essentially all determinations are made by the indirect method

For indirect calorimetry it is possible to use either a closed or an open system The first thorough work with indirect calorimetry (Benedict and Talbot 1914) was done with a closed system and most later investigators have used this system as a pattern but have modified it in varying degrees in order to overcome the difficulties present in such methods when working with infants All the closed methods are alike in principle differing only in details and most have yielded about the same results as those obtained by Benedict and Talbot My intention here is to describe in detail the method we have worked out in the Pediatric Clinic of the Caroline Institute in Norrtulls Hospital and then on the basis of work done with that method to discuss the measurement and evaluation of basal metabolism in infants

Method Since we have had its practical clinical use in mind we have tried to make our method as simple and as cheap as possible We chose a Krogh spirometer—the same apparatus usually used in Sweden for measuring basal metabolism in adults—and added to it a respiration chamber consisting of a plastic hood standing in a water seal and a bellows type circulation pump with a capacity of about 12 liters per minute The total volume of this closed system (Figures 107 and 108) is about 55 liters Since the CO is

movements In our method the water in the seal readily responds with waves to the child s movements and the kymograph curve of the volume immediately becomes uneven Also the nurse who observes the child constantly during the determination records the extent of the child s activity This record is kept on the same paper on which the volume changes with time are later superimposed providing on one sheet all the data needed Finally it is more important to have strict requirements for keeping the apparatus in perfect order for the examination of infants than for the examination of older children or adults

Basal Conditions Everybody is agreed that the child must be quiet Basal metabolism is usually determined in older children and adults in the early morning with the subject fasting to avoid the so-called specific dynamic action of food which is highest 1 to 2 hours after a meal However if an infant is fasting he usually does not lie still Most investigators seem to compromise Some give the child a low-calorie meal about one hour before the determination and determine the metabolism under conditions about 5 to 10 per cent above basal Other investigators have chosen to determine basal metabolism with the infants fasting after giving them a small amount of barbiturate We have studied infants in the morning at least three to four hours after the last meal and have given them a fast acting barbiturate (Evipan) about 15 minutes before the determination The dose of barbiturate is sufficiently low so that during the examination the child can easily be awakened if one taps lightly on the hood and at the end of the determination the child is awake A decrease in the basal metabolism has been observed only in those patients given at last 3 to 4 times our usual dose

We try to have the temperature in the chamber between 22 and 25° C Determinations done with a lower temperature show somewhat increased oxygen consumption and the same is true with temperatures of 28 to 30 C

The plan for determination of basal metabolism at our hospital is shown in Table 24

The random error of the method is about ± 4 to 5 per cent as calculated from the differences between two examinations in each of 60 infants This probably includes among other factors movements by the child that may have passed unnoticed during the observation period

STANDARD VALUES IN RELATION TO DIFFERENT BODY MEASUREMENTS To find the standard values for infants in Sweden we examined about 150 healthy infants These children all under 1 year had body weights between 1 9 and 10 kg The oxygen consumption was related to various body measurements

There is first a positive relationship between O_2 consumption and age but

in amount that introduced into the system by the child This means therefore that there will be a lapse of some time after introduction of the child into the system before this equilibrium is reached the length of time depending upon several factors The most important is the physical state of the child On the basis of temperature records in the respiration chamber in the spirometer in the rectum and on the skin of the subjects and of humidity records in the respiration chamber from about 80 determinations

Fig 109 Typical spirometer curves with record obtained from a six month old infant (Karlberg P *Acta paediat* 41 [suppl 89] 38 1952)

we have found the equilibrium time for our method to be about 30 minutes When one remembers that the child himself usually takes about 10 to 15 minutes to settle down and reach his own equilibrium 30 minutes is not unreasonably long Since the O_2 consumption is for purposes of increased accuracy recorded for 30 minutes the whole procedure takes about one hour

It is important in the determination of basal metabolism to register the child s movements in a satisfactory manner This has been done by different investigators in different ways For instance some have recorded the child s movements on a kymograph or have directly registered the child s respiratory

movements In our method the water in the seal readily responds with waves to the child s movements and the kymograph curve of the volume immediately becomes uneven Also the nurse who observes the child constantly during the determination records the extent of the child s activity This record is kept on the same paper on which the volume changes with time are later superimposed providing on one sheet all the data needed Finally it is more important to have strict requirements for keeping the apparatus in perfect order for the examination of infants than for the examination of older children or adults

Basal Conditions Everybody is agreed that the child must be quiet Basal metabolism is usually determined in older children and adults in the early morning with the subject fasting to avoid the so-called specific dynamic action of food which is highest 1 to 2 hours after a meal However if an in fant is fasting he usually does not lie still Most investigators seem to com promise Some give the child a low-calorie meal about one hour before the determination and determine the metabolism under conditions about 5 to 10 per cent above basal Other investigators have chosen to determine basal metabolism with the infants fasting after giving them a small amount of barbiturate We have studied infants in the morning at least three to four hours after the last meal and have given them a fast acting barbiturate (Evipan) about 15 minutes before the determination The dose of barbitu rate is sufficiently low so that during the examination the child can easily be awakened if one taps lightly on the hood and at the end of the determination the child is awake A decrease in the basal metabolism has been observed only in those patients given at last 3 to 4 times our usual dose

We try to have the temperature in the chamber between 22 and 25° C Determinations done with a lower temperature show somewhat increased oxygen consumption and the same is true with temperatures of 28 to 30 C

The plan for determination of basal metabolism at our hospital is shown in Table 24

The random error of the method is about ± 4 to 5 per cent as calculated from the differences between two examinations in each of 60 infants This probably includes among other factors movements by the child that may have passed unnoticed during the observation period

STANDARD VALUES IN RELATION TO DIFFERENT BODY MEASUREMENTS To find the standard values for infants in Sweden we examined about 150 healthy infants These children all under 1 year had body weights be tween 1 9 and 10 kg The oxygen consumption was related to various body measurements

There is first a positive relationship between O consumption and age but

TABLE 24

Age	0–2·3 months	2·3–6 months	6 months
Time	In the morning	In the morning	
Food	Low calorie feeding 3 hours before determination		Fasting
Sedative (Evipan)	—	About 0·01–0·02 gm./kg body weight	

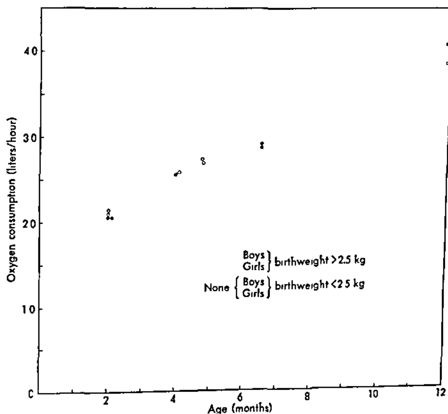


Fig. 110 The relationship between the oxygen consumption in liters per hour and age in months

among small infants especially there are large deviations (Fig 110) Differences in birth weights are probably important here

The correlation with body length is better (Fig 111) and with body weight it seems still better (Fig 112)

Oxygen consumption per kilogram of body weight decreases as body weight increases This tendency is pronounced in older children showing that the body weight cannot be used as a correlation factor for comparison between children of different ages For children of the same age it is however useful

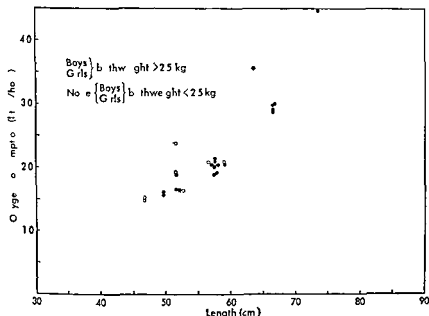


Fig 111 The relationship between the oxygen consumption in liters per hour and body length in centimeters

I have chosen to give the basal metabolism here in liters per hour In order to compare our values with previous values for infants I have also calculated them in calories per 24 hours using a value of 4 825 calories for each liter of oxygen consumed In Figure 113 those are given together with Levine s linear expressions of the standard mean values

The body weight is useful as a correlation factor for each age when we have children with normal body constitution But for abnormally thin or abnormally fat children it would be better to use a correlation factor more representative of the actual body size of the child For many years the body

surface area as calculated according to Du Bois linear formula or length weight formula has been commonly used The length weight formula is most often used because the linear formula needs so many varied body measurements Du Bois length weight formula is valid for adults and for children with body weights over 20 kg Hannon worked out a nomogram for infants according to Du Bois length weight formula From what we have been able to find in the literature, Hannon checked this nomogram by direct determination in only one child That was a child of 21 months of

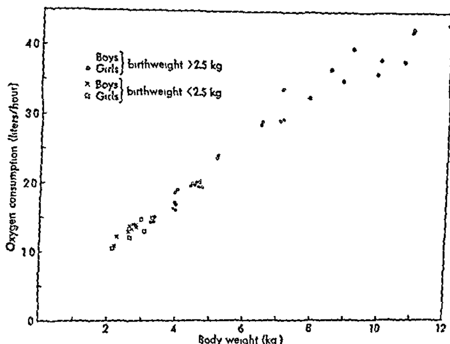


Fig 112 The relationship between the oxygen consumption in liters per hour and body weight in kilograms

age with a body weight of 6 3 kg the average weight of an infant of 4 months That child cannot have had normal body development which suggests that the nomogram may in fact be inaccurate

Edith Boyd has also given a length weight formula for calculation of the body surface It is based on about 300 direct measurements of fetuses children and adults as collected from the literature and so the measurements must have been taken from the reports of various workers who used different measurement methods

It has seemed to us to be definitely desirable to check these formulas We

have used an electrical capacity method for determination of the body surface or size In this method the child is placed on an insulated frame in the middle of a room the ceiling walls and floor of which are covered with grounded netting wire The arms and legs are extended and held at angles of about 45 degrees to the body axis The child s body surface and the wire netting form a condenser whose capacitance dependent on the size and shape of the surfaces varies only with the size of the body surface of the child in

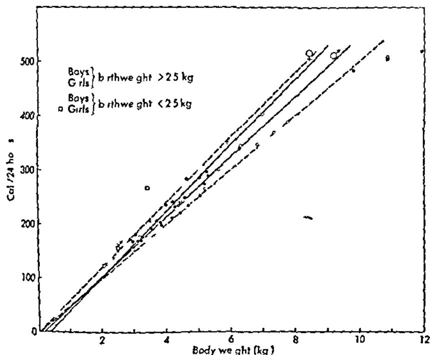


Fig 113 *The relationship between the basal metabol sm in calories per 24 hours* and body weight in kilograms Levine s standard values are introduced as regression lines for boys (♂) and girls (♀) The two broken re gression lines are 40 cal /24 hr /kg and 50 cal /24 hr /kg

determinations in the same room The capacitance is determined by the difference in adjustment of a variable condenser when high frequency alter nating current (500 000 cycles per second) is used in a resonating circuit with and without the child connected The examination takes about five minutes is simple and can be performed by a nurse Calibration consists of measuring the capacity of a number of spheres of varying sizes with electrically conducting surfaces

To make comparisons between this and older methods we examined about 200 infants and about 150 older children up to puberty. The diagram in Figure 114 shows the relationship between the capacitance surface and the body surface area as calculated according to Du Bois length weight formula. There is close agreement among the older children from puberty down to about 6 years of age but in the smaller children there is a marked difference between the results of two methods. This is greatest in the smallest infants where the Du Bois formula gives higher values.

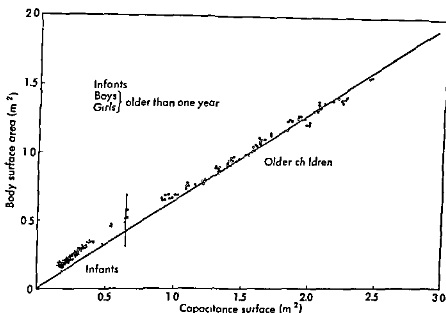


Fig 114 The relationship between the body surface area calculated according to Du Bois length weight formula and the determined capacitance surface

There seems to be no reason to assume that results with the capacitance surface method vary inaccurately in children of different size. Therefore since we know that the Du Bois formula is well worked out for older children and that there its results agree well with the capacitance surface it seems likely that the body surface according to Du Bois formula is too high for the infant period. The relationship to Edith Boyd's formula is about the same but the difference is not as great. Considering the two formulas (Boyd's and Du Bois) and knowing that the ratio between weight and height changes so much during growth (see Table 25) it seems difficult to understand how both could be expected to give correct values for the whole growth period from birth to adult life.

However even if the determination of the capacitance surface is accurate simple and brief it cannot be used for clinical use Therefore we have worked out an empirical nomogram with which the capacitance surface can be calculated from the weight and length

Figure 115 shows the relationship between oxygen consumption from our basal metabolism material and body surface as calculated according to Du Bois formula The correlation is good but the extrapolated line expressing mean O_2 consumption does not go to zero This may be due to the fact that the formula gives relatively higher values of S for smaller infants than for older infants The capacitance surfaces of the infants in our material have

TABLE 25

Age year	Height cm	Weight kg	H W
0	50	3 5	14 3
½	65	7 7	8 4
1	77	10	7 7
5	110	18	6 1
10	139	31	4 5
15	165	54	3 1

Du Bois
$S = 71\ 84\ W^{0\ 42}\ H^{0\ 725}$
Edith Boyd
$S = 3\ 207\ W^{73 - 0 \ W}\ H^{0\ 3}$

been calculated from our nomogram and Figure 116 shows capacitance surface as the correlation factor for basal metabolism Here the line expressing mean O_2 consumption when extrapolated goes much nearer to zero and the deviations are smaller than for instance when body weight is used as the correlation factor The fact is that the accuracy of the capacitance surface method is not as greatly affected by variations in the body constitution as is the accuracy of either the Du Bois or the Edith Boyd formula Since the material is not yet worked out statistically I cannot now give the deviations in figures

In Figure 116 several cases of hypothyroidism untreated and treated are included

So far I have been talking about the determination of basal metabolism in infants using a closed system which is by far the most common The prin

ciple of methods using an open system which I want to mention only briefly is that the child breathes an air stream moving with known speed and the *differences in concentrations of O and CO* in the air, before and after passing the child are determined These analyses are done with a Haldane apparatus or with a more intricate technical apparatus Variations in the O_2 consumption and the CO output can also be determined with shorter observation periods with the open system

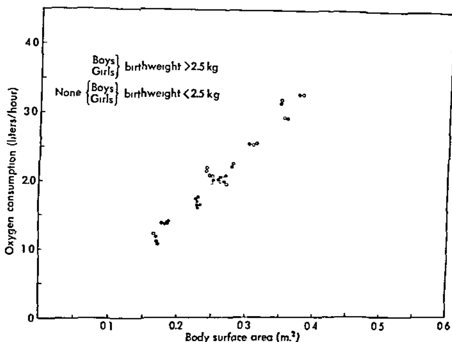


Fig 115 The relationship between the oxygen consumption in liters per hour and the body surface area calculated according to Du Bois length weight formula

Recently I have started working with such a method I have not yet had enough experience however to discuss it

Professor RÄIHÄ (Helsinki) In Helsinki we have been interested in the basal metabolism of prematures Dr Malm is using an apparatus built of a plastic tube in which we put the infant The inflow gas can be atmospheric air or a mixture of oxygen and air The oxygen content of the outflow gas is estimated by an oxygen analyser constructed by Dr Malm and members of our institute of technology We can simply read the oxygen content of the gas mixture coming from the chamber

Figure 117 shows the oxygen used by a 1100 gm premature at first in air and then in a 50 per cent oxygen mixture

In Figure 118 we have a 10 day-old child of the same size and in the first column we see the basal metabolism in air and in the second in a 44 per cent oxygen mixture You can see that we get a rise I cannot explain that in ny other way than that this child has an oxygen debt during its life in an air atmosphere It is living as we would live if we had to run a 100 meter race

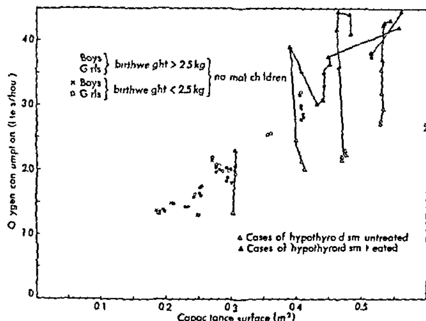


Fig 116 The relationship between the oxygen consumption in liters per hour and the capacitance surface

We could find no difference in oxygen saturation in the blood in this infant in air or in 60 per cent oxygen

Professor BARNETT (New York) I should like to ask Professor Raihà whether in babies in whom there is an increase in basal metabolism when the oxygen in the atmosphere is increased one can find also a difference in oxygen capacity I ask this because there are a number of observations made by Nelson Ordway at Louisiana State University using the oximeter in which he was able to show that anoxic premature infants could be divided into groups one showed an increase in oxygen saturation when breathing a higher oxygen atmosphere and the other group showed no increase

Professor RAIHA (Helsinki) We have one premature who is a blue baby and in that one there is a definite difference *between the oxygen saturation* in atmospheric air and in an oxygen incubator

Professor LEVINE (*New York*) *I* think that Dr Karlberg has devised a

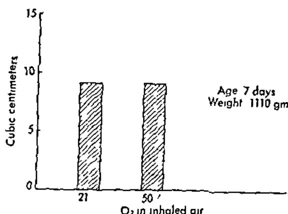


Fig 117 Oxygen consumption per kilogram of body weight

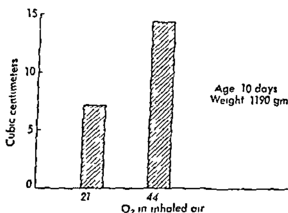


Fig 118 Oxygen consumption per kilogram of body weight

very ingenious method for determining surface area The problem of course has been one that has intrigued physiologists for a long time To my knowledge Dr Karlberg s technique is the first application of electrostatics to the measurement of surface area of infants

With regard to determining standard metabolism what is the magnitude of the error if one determines the calories consumed and the oxygen used with

out knowing the respiratory quotient? I think that with oxygen if you assume a respiratory quotient of 0 82 the error is less than 3 or 4 per cent but I do not remember the figures Second is it fair to compare the standard metabolism of babies under 6 months who receive no barbiturate with infants over 6 months who are given small doses of barbiturate?

Dr KARLBERG (Stockholm) For the calculation of the calories produced in 24 hours on the basis of oxygen consumption of the child we have used a respiratory quotient of 0 86 the mean value of Benedict and Talbot s investigations I cannot give a figure for the error involved in doing this since our method is worked out for practical clinical use and we only determine the oxygen consumption

In answer to the second question barbiturate is given to infants of 2 to 3 months of age The dose chosen is so low that it cannot perceptibly influence the basal metabolism as determined by our method Oxygen consumption decreases only after giving doses at least two or three times as great as those we give

Professor PLUM (Copenhagen) I have done some metabolic studies on children down to 4 years of age and when they slept the basal metabolism dropped about 15 per cent below their waking value The question of food is not very important I think if they are only given a small meal I estimated the basal metabolism with and without a small meal beforehand some years ago and found no difference

It is a pity that Dr Vogelius of Copenhagen is not here because he has written a treatise describing the great difficulties of the problem you mentioned He found that it was nearly impossible to estimate the basal metabolism if the child does not correspond to normal values according to age height and weight Vogelius did not even find that the surface area gave good correlation since he could not determine how to account for an abnormal shape

Have any comparisons of the capacitance surface and direct measurement of the surface area been made?

Dr KARLBERG (Stockholm) I have worked only with infants and they are not as susceptible to emotional disturbance from the investigation as are older children The infants were either restless and so were not included in the material or they were motionless and so made it difficult for us to know whether they were sleeping or awake The possible difference in oxygen consumption between children in these two similar states must therefore be included in the random error of the method as calculated from the differences between duplicate determination in each of many infants The random error of the method has been found to be ±4 to 5 per cent

Boys
Girls

Heart volume (cc)

Body surface area (m²)

Fig 119 The correlation between heart volume and body surface area (according to Du Bois length weight formula) in normal infants (Lind J *Acta radiol* suppl 82 p 94 1950)

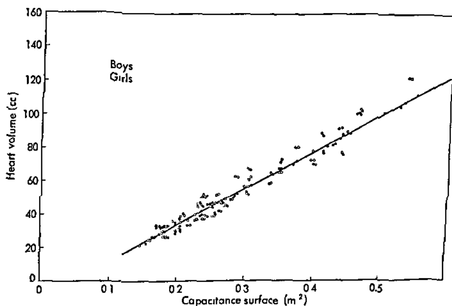


Fig 120 The correlation between heart volume and the capacitance surface (Lind J *Acta radiol* suppl 82 p 107 1950)

Regarding the electrical method of determining the body area we tried at first to do direct determinations of the body surface area using different methods All of those however seemed to have possible inherent error

It is doubtful whether it really is the total geometrical body surface area which we want as a correlation factor What we need is a body measurement *representing the size of the body better than age height and weight* separately or collectively The electrical capacitance method gives a meas*ure of the body surface area which is exposed to the surroundings* but it is also influenced to a certain extent by the bodily proportions of the child *The capacitance surface is given in square meters* though it is only a relative measure which we have introduced as a correlation factor because of its *apparent relative accuracy in individuals of different ages*

Dr Lind at the Norrtulls Hospital has made determinations of heart volume roentgenologically in about 300 infants and we have related these values to capacitance surface

Dr LIND (Stockholm) Figure 119 relates the Du Bois surface area to heart volume and there is a correlation of 0 95 In Figure 120 it can be seen that heart volume has an even closer correlation 0 99 to the capacitance surface and the regression line extrapolates closer to zero than in Figure 119

Dr KARLBERG (Stockholm) We have also determined the blood volume of infants with the carbon monoxide method and the diagram in Figure 121 shows the relationship of blood volume to capacitance surface

Since the relationships between the capacitance surface and (1) the basal metabolism (2) the heart volume and (3) the blood volume seem to be about the same there must be good correlation among these three also

Dr HAGEDORN (Copenhagen) I would like to ask the capacity of *the pump*

Dr KARLBERG (Stockholm) The capacity of the pump is variable *but usually we use about 32 liters per minute The exact value is not so im*portant but it is essential that the pump always run at the same speed during the whole determination If the pump speed changes the gas equilibrium in the system immediately disappears

Professor LEVINE (New York) If the capacitance varies slightly with the composition and shape of the body does it vary with the normal changes that take place in the water and electrolyte contents of the body from birth to one year of age?

Dr KARLBERG (Stockholm) Since we use such a high frequency alternating current 500 000 cycles the weak electric stream just follows the surface of the skin We have determined that normal changes in the skin have no influence on the measurements

Professor BARNETT (New York) As I understand it demonstrating a correlation between a number of functions such as blood volume heart volume and oxygen consumption with a measure such as capacitance surface as you have done does not in itself validate this technique as an absolute measure of anything The carbon monoxide method for measuring blood volume is open to the errors of any red cell method of measuring blood volume caused by uneven distribution in different parts of the body If a plasma method for determining blood volume is used instead of a red cell

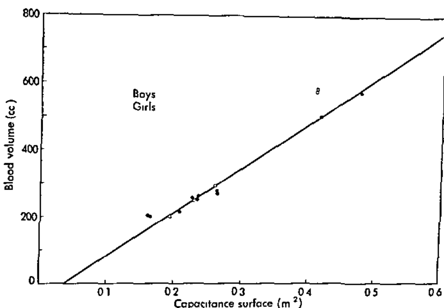


Fig 121 The relationship betw en the blood volume in cubic centimeters and the capacitance surface

method quite different figures are obtained which might correlate with an other variable such as surface area

Dr KARLBERG (Stockholm) While I cannot be sure that I have an absolute measure of blood volume I have at least a relative value And when we relate it to a second factor such as capacitance surface it does not matter whether it is a relative or absolute

Professor BARNETT (New York) My point was that the demonstration of a good correlation between A B C and D does not necessarily mean that D is an accurate measure of the others It only means that it changes in the same way as the others do

Dr KARLBERG (Stockholm) Yes possibly so But we must consider that all of these physiological factors vary from the same primary cause if all of them vary together If one factor varies independently of the others there would seem to be good reason to suspect inaccuracy

REFERENCE

Karlberg P Determinations of Standard Energy Metabolism (Basal Metabolism) in Normal Infants *Acta paediat* 41 Supplement 89 1952

CHAPTER 11

Panel on Metal Metabolism

IRON METABOLISM

Professor VAHLQUIST (Uppsala) The following review deals to some extent with investigations of my own and to some extent with the results of other workers I *shall treat the problem of iron metabolism in infancy* mainly from a clinical point of view and focus attention on the serum iron since I myself have studied this fraction for quite a long time

It has been known since the middle 30 s that in the plasma of the blood there is a minute fraction of acid soluble iron normally amounting to about *1 mg of iron per liter of plasma This special fraction of iron deserves great* attention since it represents the transport form of iron within the body Carefully interpreted results of determinations of the serum iron fraction will give some insight into iron metabolism

I should like to consider first the physiological changes in serum iron and hemoglobin at various ages In the neonatal period infants have very high hemoglobin between 18 and 20 gm per cent In the following months there is a steep fall in hemoglobin and then the level remains fairly stable until school age when it slowly rises At puberty there is a definite rise although almost exclusively in males

On the whole serum iron follows the hemoglobin curve but there are differences For instance during the first one or two days of life there is a sharp drop in serum iron After this the serum iron shows a transient increase before starting a slow and progressive downward slope (Fig 122)

Figures 123 and 124 show in schematic form the situation in mother and fetus during pregnancy and in the first two weeks following delivery

The hemoglobin of the pregnant mother declines especially in the second half of gestation because of hydremia

Beginning with the investigations of Wintrobe and collaborators early

fetuses have been shown to have low hemoglobin values These observations were corroborated in my thesis of 1941 In early human fetuses one may find values as low as 5 gm per cent or less Several months before birth the final concentration is eached Hence one will find that premature children are born with hemoglobin values which are as high as those in children born at term

It is evident that there is not only an absolute increase in the hemoglobin of the fetus but that there is also a relative increase until the last trimester since the concentration steadily rises

In some respects serum iron reflects the hemoglobin curve In the preg nant woman in the second half of gestation there is a slight decline in serum

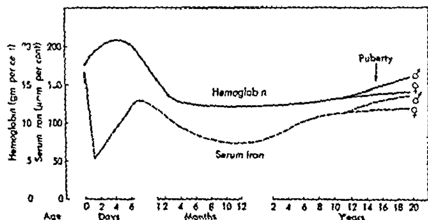


Fig 122 Hemoglob n and serum iron in relation to age

iron which levels off by the ninth month and just before delivery there may be a slight increase Following delivery there is a fairly regular drop during the first day

Low values are found early in gestation in the fetus but in the last trimester serum iron increases until at birth the levels are definitely above those of the mother Average values are 160 μgm per cent for infants and 100 μgm per cent for mothers This difference is found also in children born after Cae sarean section where birth trauma was avoided

In later infancy and in the second or third years serum iron is at a com paratively low level The question arises whether these moderately low serum iron values are due to iron deficiency To find out I performed an experiment some years ago in collaboration with Dr Moller There were 25 children aged 6 to 8 months who had had no iron treatment previously All

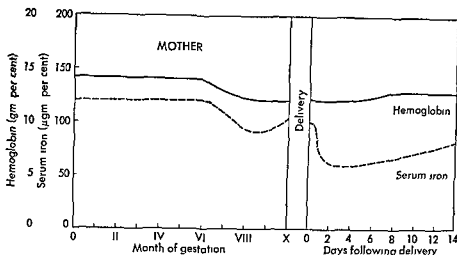


Fig 123 Hemoglobin and serum iron values of the mother during pregnancy and immediately following delivery

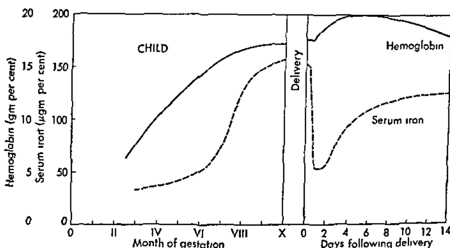


Fig 124 Hemoglobin and serum iron values of the child during fetal life and in *the newborn child*

were full term Before iron treatment mean serum iron was 87 µgm per cent hemoglobin was 12 gm per cent and erythrocytes were 4 3 million per cubic millimeter Following six weeks of ferrous iron treatment of about 250 mg a day the following values were obtained 88 9 µgm per cent 12 gm per cent and 4 5 million per cubic millimeter *Thus there was no* change whatsoever Hence it does not seem likely that the moderately low values for serum iron and hemoglobin *in this period are due to iron deficiency*

These results are at variance with the observations of Dr Mackay She found iron therapy distinctly effective It should be stressed however that the hemoglobin values in my patients before treatment were at the same level as those obtained by Dr Mackay after iron treatment So evidently there are differences in various populations In Sweden in the urban population there does not seem to exist a widespread iron deficiency at an early age

It has been claimed that iron deficiency is more prevalent among artificially fed children than among those breast fed This is not confirmed by the findings in the investigation just cited These children showed just the same values for hemoglobin and serum iron in both groups

It is known that the serum iron concentration normally fluctuates during the day The fluctuations are not quite regular but there is a definite trend toward higher values in the morning and lower values in the evening I pointed this out in 1941 and in the past year I have taken up the problem again in collaboration with Dr Hellstrom to determine at what age this rhythm appears We have investigated infants in the first trimester of life and at this time the tendency toward daily rhythm is less pronounced than later We all know that fluctuations in body temperature are at a minimum during early life and this is another indication that rhythmical processes are not mature so to speak at this period

These observations may have some bearing on the problem of feeding children If rhythmical processes are not developed during the first six weeks of life it is perhaps not so surprising that the child cannot always tolerate an 8 hour interval at night between meals

In premature children there is the same decrease in the hemoglobin after birth which occurs in full term children but it is a little more marked Furthermore following the initial fall there is a progressive anemia which has the characteristics of iron deficiency with the erythrocyte values almost constant while the hemoglobin values decline hence giving rise to microcytosis and hypochromia These changes are reflected in the serum iron which eventually decreases to very low values

Figure 125 gives a typical picture of the developments Thus the premature child develops an early normochromic anemia which is iron resistant and a later hypochromic anemia which is definitely responsive to iron therapy

Finally some comments on total iron from the work of Professor McCance of Cambridge At birth the average content of iron in the liver is 38 mg and there are 235 mg of iron in the hemoglobin At about six months of age the corresponding values are 26 and 389 mg or an increase of the iron in the hemoglobin of 154 mg Of this 154 mg only 12 mg come from the liver Hence the bulk of the iron accumulated during the first six months of

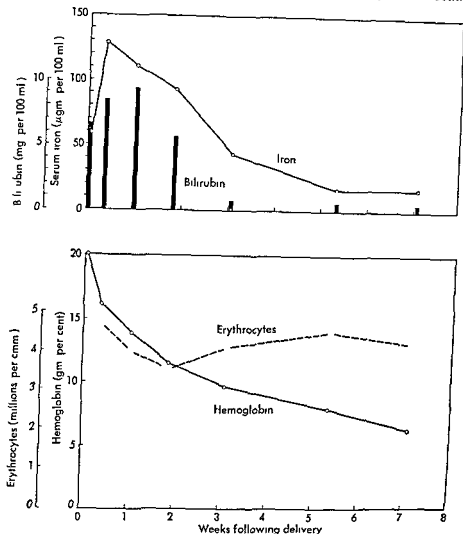


Fig 125 Hemoglobin and serum iron values during the development of iron deficiency anemia in a prematurely born child Birth weight 1770 gm

life must come down from other sources and the liver depot of Bunge seems of little value for hematopoiesis

Other sources of iron are found in food The iron in the food at this early age comes primarily from milk We know from investigations of Dr Wallgren and others some 20 years ago that the concentration of iron in breast milk is about 0 5 mg per liter In cow s milk it may be even lower than that By adding cereals and solids however the final iron content may not be lower

than in human milk alone That may be the explanation why there was no difference in iron levels in the serums of the breast fed and artificially fed children I described

THE RATE OF INCORPORATION OF RADIOACTIVE IRON INTO HEMOPROTEINS AND FERRITINS

Professor THEORELL (Stockholm) These experiments have been carried out by a team at Biokemiska avdelningen Medicinska Nobelinstitutet including Margit Beznak Bonnichsen Paul Åkeson and myself The investigation was financially supported from Medicinska forskningsrådet and Stiftelsen Therese och Johan Anderssons minne

Guinea pigs proved to be a suitable species for our work since they offered certain definite advantages for the preparative work especially in the case of the catalases The animals were injected intraperitoneally with 0 05 mg radioactive iron per 100 gm body weight (as ferric ammonium citrate) At intervals they were sacrificed about 15 animals being taken for each determination The blood was used for the preparation of hemoglobin and blood catalase The hind leg muscles were perfused to free them from blood and the muscle used to prepare myoglobin Livers and spleens were taken for the preparation of ferritins and catalase The muscles except what had been removed for the myoglobin preparations were taken for cytochrome-c preparation The rest of the animal was analyzed separately for iron and radioactivity

The total iron content of guinea pigs was found to be 5 95 mg per 100 gm wet weight Blood free liver contained 6 4 mg per 100 gm wet weight

For the determinations on hemoglobin we simply used washed red blood cells since in experiments of this kind the nonhemoglobin iron in them is quite negligible compared to the hemoglobin iron The other hematin proteins are present in minute amounts and have to be purified with more or less elaborate procedures before their iron can be obtained free from inert contaminants

Myoglobin the content of which is about 0 03 per cent in the fresh muscles of guinea pigs was eluted with water Inert proteins were removed by means of lead acetate precipitation and the remaining solution fractionated with ammonium sulphate at different degrees of saturation Thus three fractions obtained as precipitates when the salt concentration was gradually increased were obtained and labeled "70–80" "80–90" and "90–100" the figures referring to the per cent of saturation with salt By spectrophotometry it was found that all fractions were contaminated with hemoglobin but

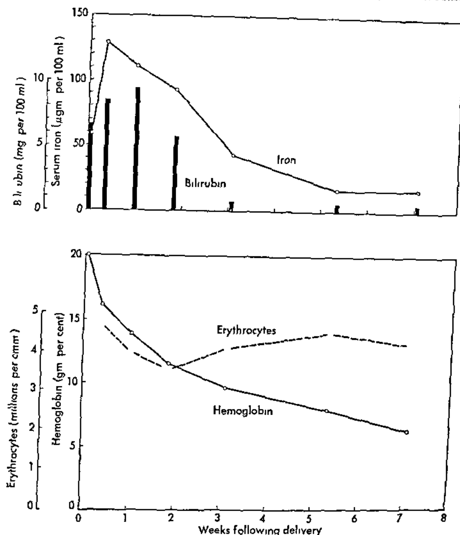


Fig 125 Hemoglobin and serum iron values during the development of iron *deficiency anemia in a prematurely born child Birth weight 1770 gm*

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Other sources of iron are found in food The iron in the food at this early age comes primarily from milk We know from investigations of Dr Wallgren *and others some 20 years ago that the concentration of iron in breast* milk is about 0 5 mg per liter In cow s milk it may be even lower than that By adding cereals and solids however the final iron content may not be lower

than in human milk alone That may be the explanation why there was no difference in iron levels in the serums of the breast fed and artificially fed children I described

THE RATE OF INCORPORATION OF RADIOACTIVE IRON INTO HEMOPROTEINS AND FERRITINS

Professor THEORELL (*Stockholm*) These experiments have been carried out by a team at Biokemiska avdelningen Medicinska Nobelinstitutet including Margit Beznak Bonnischsen Paul Ake on and myself The investigation was financially supported from Medicinska forskningsradet and Stiftelsen There e och Johan Anderssons minne

Guinea pigs proved to be a suitable species for our work since they offered certain definite advantages for the preparative work especially in the case of the catalases The animals were injected intraperitoneally with 0 05 mg radioactive iron per 100 gm body weight (as ferric ammonium citrate) At intervals they were sacrificed about 15 animals being taken for each determination The blood was used for the preparation of hemoglobin and blood catalase The hind leg muscles were perfused to free them from blood and the muscle used to prepare myoglobin Livers and spleens were taken for the preparation of ferritins and catalase The muscles except what had been removed for the myoglobin preparations were taken for cytochrome-c preparation The rest of the animal was analyzed separately for iron and radioactivity

The total iron content of guinea pigs was found to be 5 95 mg per 100 gm wet weight Blood free liver contained 6 4 mg per 100 gm et weight

For the determinations on hemoglobin we simply used washed red blood cells since in experiments of this kind the nonhemoglobin iron in them is quite negligible compared to the hemoglobin iron The other hematin proteins are present in minute amounts and have to be purified with more or less elaborate procedures before their iron can be obtained free from inert contaminants

Myoglobin the content of which is about 0 03 per cent in the fresh muscles of guinea pigs was eluted with water Inert proteins were removed by means of lead acetate precipitation and the remaining solution fractionated with ammonium sulphate at different degrees of saturation Thus three fractions obtained as precipitates when the salt concentration was gradually increased were obtained and labeled 70–80 80–90 and 90–100 the figures referring to the per cent of saturation with salt By spectrophotometry it was found that all fractions were contaminated with hemoglobin but

to a decreasing extent with increasing salt concentration The ratios of myo globin to hemoglobin were measured spectrophotometrically and from the total amount of iron in a sample its specific activity and the specific activity of the hemoglobin obtained from the blood the specific activity of the myoglobin in each of the three fractions was calculated (Table 26) You will see that the correction for the hemoglobin decreases toward higher salt saturations and also that the three values for the myoglobin activity agree fairly well

Cytochrome c was prepared from muscle by extracting it with dilute sul phuric acid fractionating the neutralized extract with ammonium sulphate and dialyzing against dilute ammonia Since the preparation thus obtained was always found to contain some noncytochrome iron a procedure devel oped by Paul was employed for its further purification This procedure con

TABLE 26

Correction of Myoglobin Preparations for Their Hemoglobin Contents*

Fraction	Myoglobin hematin in per cent of total hematin	Specific activity		
		Total	From Hb	From Mgb
70 80	47 0	0 155	0 110	0 045
80-90	69 5	0 105	0 064	0 041
90-100	97 5	0 052	0 005	0 047
Hemoglobin	—	—	0 209	—

* Modified from Theorell Béznak Bonnichsen Paul and Åkeson

sists of the removal of all hematin from the myo and hemoglobin which may be present with acid acetone the splitting of the thio ether bonds between the protein moiety and the porphyrin skeleton in cytochrome c with silver salts at slightly acid reaction the extraction of the cytochrome c hematin with acid acetone from the reaction mixture and the final purification of the hematin by washings of its butanol solution with hydrochloric acid and water This procedure was tested on artificial mixtures of radioactive hemoglobin and fer ritin with inactive cytochrome c In those mixtures the conditions for ob taining inactive cytochrome-c iron were much less favorable than in the preparations obtained from guinea pigs Nevertheless a good purification was achieved (Table 27)

Catalases from liver and blood were prepared according to ordinary meth ods employing chloroform-ethanol treatment of the aqueous extracts fol lowed by ammonium sulphate fractionations and dialysis The hematin was released from the catalase proteins by acid acetone and finally washed with

hydrochloric acid and water to remove traces of foreign iron In this case too tests were made with artificial mixtures

Ferritin was obtained by ammonium sulphate fractionations of the super natants after heat coagulation of inert material in water extracts of livers and spleens

The amounts of iron dealt with in these procedures were in some cases ex tremely small Thus the yield of blood catalase iron ranged between 4 and 28 µgm This means that every possible precaution had to be taken to pre vent entry of foreign iron from reagents or contamination a rather difficult task since the material had to pass through five various steps after its prepara tion before the final value could be obtained

TABLE 27

Isolation of Cytochrome c Iron from Mixtures of Cytochrome c (inactive) Hemoglobin (radioactive) and Ferritin (radioactive)*

Mixture contained			Yield of cy c Fe per cent	Net c p m in isolated iron
µgm iron				
from cy c	total	c p m		
46 5	484 9	3773	53 3	2 2
23 3	461 7	3773	72 1	1 5
46 5	162 5	989	74 4	2 0

Modified from Theorell Béznak Bonnichsen Paul and Åkeson

From Tables 28 and 29 you can see the distribution and the recovery of the radioactivity in some organs after various times Quite conspicuous is the high liver value on the second day Normally drugs administered intra peritoneally are absorbed very rapidly so that the blood level almost immedi ately reaches the same value as found after intravenous administration Hahn and Granick found that after 1 hour 40 per cent and after 2 hours 60 per cent of intravenously given iron had been deposited in the liver Thus the absorption of iron from the peritoneum must be comparatively slow This agrees with the high value for Rest of animals on the first day since that part includes peritoneum and the contents of the peritoneal cavity Ob viously the spleen does not take part in the iron metabolism to the same ex tent as does the liver The values for the red blood cells increase slowly toward a final value as could be expected This final value seems to be the

TABLE 28

Distribution of Radioactivity Series 2*

Organ	Per cent	Days after injection 1	2	3	4	6
Liver	Sp act	3.72	6.01	3.85	4.52	4.16
	Recovery	28.4	34.4	20.5	32.1	27.4
Spleen	Sp act	0.26	0.92	0.65	0.49	0.85
	Recovery	0.3	0.5	0.5	0.5	0.4
Red blood cells	Sp act	0.09	0.23	0.29	0.27	0.50
	Recovery	2.6	5.4	8.5	7.3	16.8
Muscle for cy c	Sp act	0.45	0.56	0.36	0.13	0.53
	Recovery	14.6	13.6	11.4	12.7	14.8
Muscle for mgb	Sp act	0.27	0.61	0.52	0.27	0.49
	Recovery	0.4	0.5	0.5	0.4	0.5
Rest of animals	Sp act	0.81	0.46	0.51	0.49	0.52
	Recovery	45.0	31.7	33.1	28.7	24.5

* Modified from Theorell Béznak Bonnichsen Paul and Åkeson

TABLE 29

Distribution of Radioactivity Series 1*

Organ	Per cent	Days after injection 7	14	21	28	35
Liver	Sp act	3.45	2.66	1.25	1.38	1.24
	Recovery	20.3	16.2	8.2	10.2	9.2
Spleen	Sp act	0.23	0.26	0.19	0.22	0.31
	Recovery	0.2	0.3	0.2	0.2	0.3
Red blood cells	Sp act	0.15	0.35	0.27	0.63	0.65
	Recovery	4.1	8.5	7.0	17.9	15.9
Muscle for cy c	Sp act	0.09	0.21	0.16	0.26	0.20
	Recovery	5.8	4.3	3.7	6.4	9.3
Muscle for mgb	Sp act	0.18	0.20	0.12	0.36	0.37
	Recovery	0.2	0.2	0.1	0.4	0.3
Rest of animals	Sp act	0.31	0.19	0.15	0.32	0.46
	Recovery	17.9	9.3	11.1	23.3	24.0

* Modified from Theorell Béznak Bonnichsen Paul and Åkeson

same for all investigated organs about 0.5 per cent specific activity The activities of the isolated compounds arrived at the same value (Figs 126 and 127) The peaks on the second day in the specific activities of the total liver iron and the liver ferritin iron are seen The liver catalase also has a pronounced maximum although somewhat delayed as compared to the other

two The difference is quite obvious between the liver and the blood catalases The latter follows hemoglobin closely The myoglobin and cytochrome c show a very slow rate of incorporation In fact they are slow enough to permit the effect of the growth of the animals during the time of the experiments to influence the values which might explain the apparently accelerated rate of incorporation

From the results presented in Tables 28 and 29 and in Figures 126 and 127 certain conclusions can be drawn

1 Blood catalase and liver catalase are synthesized separately in spite of the fact that they have identical protein components as shown by Bonnichsen

2 Greenstein has shown that the catalase activity in liver drops to ⅓–½ of its normal value within one or two days after the implantation of a cancer in rats A few experiments which we performed on rats indicated that the rate of incorporation of radioactive iron into liver catalase was about the same for guinea pig and rat Thus the rapid fall in liver catalase activity reported by Greenstein cannot depend upon a hampered synthesis Either an accelerated breakdown or the influence of some inhibiting substance on the activity of catalase must be assumed to explain Greenstein s results

3 From English and American laboratories it has been reported that when N labeled glycine is given to man the stercobilin excreted during the following 3 to 4 months becomes N^1 labeled The values for atom per cent excess show two periods with high N^5 content in stercobilin one during the first 10 to 14 days the other beginning about 4 months later at the same time as the disintegration of the hemoglobin hematin starts The nature of the parent substances to these two waves has been discussed From our experiments we can say that liver catalase can contribute to the first wave but quantitatively only to a very limited extent (The hematin from the catalase in one guinea pig liver corresponds to the hematin content in 0 06 ml blood) Blood catalase in this respect can not be differentiated from hemoglobin Myoglobin cannot contribute to the first wave but possibly to the last one and the same is true for cytochrome c but to a quantitatively lesser extent

4 The total iron content of blood free muscle tissue was found to be 1 36 ± 0 05 mg per 100 gm wet weight To this figure hemoglobin and myoglobin contribute equally with about 0 1 mg each and cytochrome c with 0 02 mg Thus about 1 mg is left for other substances Since myoglobin and cytochrome c always showed lower specific activities than did the muscle used for myoglobin there must be some substance(s) with a relatively high specific activity in the muscl tissue In spite of the availability of precursor substances the rate of incorporation in myoglobin is low

From the investigations of Whipple et al in th United States and of Biorck

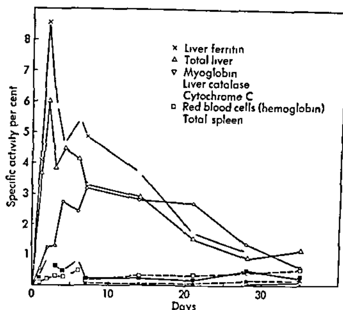


Fig 126 Specific activities of radioiron in some organs and of isolated compounds after various times (Theorell H Béznak M Bonnichsen R Paul K. G and Åkeson Å *Acta chem scandinav* 5 468 1951)

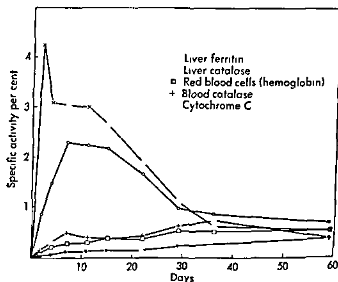


Fig 127 Specific activities of radioiron in some organs and of isolated compounds after various times (Theorell H Béznak M Bonnichsen R Paul K. G and Åkeson Å *Acta chem scandinav* 5 467 1951)

in Sweden it is known that anemia immobilization or infectious diseases do not change the myoglobin contents of muscle tissue

Further experiments are going on to determine whether the rapid turnover found for liver catalase as compared to the other hematin proteins is a property common to all liver hematin proteins

A detailed report of these investigations appears in *Acta chemica scandinavica* in Spring 1951

WHAT IS THE FUNCTION OF TRANSFERRIN IN PLASMA?

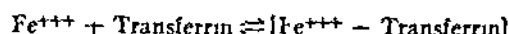
Dr LAURELL (Lund) I am going to discuss two problems the form in which iron occurs in plasma and the form in which iron enters and leaves the blood

The iron found in native plasma is almost quantitatively bound to a special protein This protein appears in the literature under the following names the iron binding component β 1 metal-combining globulin siderophilin and transferrin I will use the name transferrin today Transferrin has a much greater tendency to form complexes with iron than any other protein in serum Each molecule of transferrin can bind two atoms of iron The iron transferrin complex has a deep salmon pink color In normal individuals transferrin is only one third saturated with iron The only pathological conditions known in which transferrin is saturated or almost saturated with iron in vivo are hemochromatosis severe hemolytic crisis and severe cases of untreated pernicious anemia If these diseases can be ruled out it can be assumed that the iron concentration in serum in health and in disease is lower than the iron concentration necessary to saturate transferrin with iron

The concentration of serum iron varies in health and disease as is well known but the concentration of transferrin also varies in different conditions within rather broad limits This will not be discussed here

From what has been mentioned above it is evident that transferrin is the carrier of iron in mammalian plasma It has however not been satisfactorily ascertained if transferrin in vivo is a carrier of iron just as hemoglobin is a carrier of oxygen or if transferrin takes up and delivers iron in different organs of the body without being metabolized itself

To facilitate further discussion of this point I present a schematic expression for the reaction between iron and transferrin



$$Fe^{+++} + \text{Transferrin} \rightleftharpoons [Fe^{+++} - \text{Transferrin}]$$

An important question is whether or not this reaction is reversible in vivo

since it is known to be reversible *in vitro* The reaction proceeds however nearly quantitatively to the right at the usual pH of blood 7 3 It has therefore generally been accepted that transferrin takes up ionized iron diffusing into the blood from hemoglobin destroying organs the iron depots and the mucosal cells in the intestinal tract The iron transferrin complex called serum iron is conducted via the blood to the bone marrow and other organs where there is a need for iron for synthesis It would be very interesting to know in which form iron leaves the blood to enter the iron-consuming organs

Two different hypotheses have been put forward

1 It is assumed that the capillaries are relatively permeable to the iron protein complex and that the whole iron transferrin molecule leaves the blood This theory was advanced on the assumption that the iron transferrin complex was completely undissociated within the whole physiological pH range

2 It is assumed that an equilibrium exists in plasma between ionized iron iron free transferrin and iron transferrin The ionized iron of plasma would be in equilibrium with the iron of the extravascular fluid through simple diffusion Only the ionized iron should according to this hypothesis leave the blood stream Now the actual concentration of ionized iron in plasma must be very small When the blood passes organs where synthesis of iron-containing substances is in process the reaction as written would proceed to the left The same would be true if the pH of the blood decreases in any organ In this way the transferrin molecules could serve as real transporters of iron This hypothesis was advanced because several experimental observations were made which were difficult to interpret without it

The observations which support the latter hypothesis follow

The transferrin concentration in plasma was found to remain constant during oral and intravenous iron tolerance tests in spite of the great changes in serum iron concentration that occurred The same was true during the rapid initial decrease in serum iron which occurred when liver preparations were injected into a patient with pernicious anemia

The relation between the concentration of iron free transferrin and of iron transferrin in plasma shows regular variations under various pathological and physiological conditions It has repeatedly been shown for example that the ratio between iron transferrin and iron free transferrin is higher than normal when the depot iron increases and lower than normal when the depot iron decreases

These facts may be explained if the function of transferrin is to establish an equilibrium between ionic iron in the various organs of the body In other words the law of mass action can be applied to the reaction written above

between iron and transferrin These facts however are difficult to explain if the whole iron transferrin molecule leaves the blood

Recently further experimental data concerning intermediate iron metabolism have been collected which elucidate this problem

In the course of studies on the anemia of infection Cartwright and Wintrobe injected transferrin intravenously in such quantities that the concentration of transferrin in the plasma increased between 50 and 100 per cent The transferrin was afterwards saturated with iron by an injection of ionized iron These investigators found that the serum iron returned to its initial level within seven hours after the iron injection in spite of the increased concentration of transferrin The transferrin concentration returned to its previous level only after some days These experiments clearly show that the elimination of iron and transferrin did not proceed simultaneously

Huff and collaborators have determined the serum iron turnover by studying the turnover of injected small quantities of transferrin labeled with radio iron In normal individuals they found a turnover rate of 0 35 mg per kilogram of body weight per day In patients rapidly forming red cells much higher values were obtained The highest value found was 4 2 mg in a patient with polycythemia

If the first hypothesis that the whole iron transferrin molecule leaves the blood is correct we can transform the values of the iron turnover to values of transferrin since the molecular weight of transferrin is known If this assumption were true about 32 gm of transferrin would be metabolized per day in a normal person of 70 kg After acute loss of blood and especially in polycythemia turnover values of hundreds of grams would be obtained

The data presented are hard to reconcile with the assumption that iron leaves the blood stream as an iron transferrin complex They support however the hypothesis that iron leaves the blood stream in ionized form and that transferrin in plasma is a real carrier of iron just as hemoglobin is a carrier of oxygen

Professor SALOMONSEN (Oslo) Variations of the serum iron may range from below 25 μgm per cent at 2 A M to 200 μgm per cent during the day I should think 8 o clock in the morning is not a good time to take blood samples because then the serum iron level is rapidly increasing What is the optimal time?

Do we know the reason for this fluctuation in serum iron? Factors such as sleep food intake brain work and muscular activity may be important Hoj r in Denmark has shown that in people who work at night and sleep during the day the serum iron shows inverse fluctuations compared to the normal What about infants with repeated periods of sleep during the day cry

ing and intake of food at night? Professor Vahlquist said that in the first six weeks of life the fluctuations are not the same as later but what happens after six weeks of age?

Professor VAHLQUIST (Uppsala) This is certainly a problem of practical as well as theoretical interest When I collected my data for serum iron values in healthy children I always took the samples during the fasting state between 8 and 9 o clock in the morning and I have continued to do so There were however some instances in which it was difficult to adhere to a specific time for taking samples For instance sometimes I had to take samples from children cared for in institutions outside the hospital Under these circumstances I found values of about 60 μgm per cent for children between 6 and 8 months Later when I repeated these examinations and took samples at 8 A M I found a somewhat higher value about 80 μgm per cent However I cannot say that 8 A M is the best time but rather that the important thing is always to use the same time when you make comparisons

The rhythmical fluctuations are seemingly less pronounced in disease than in health In pernicious anemia for instance when one follows the hypersideremia with repeated examinations during the day the fluctuation is smaller than one would expect in a healthy adult

Professor Salomonsen asked about the appearance of the rhythm We have studied children one month old three months old and six to eight months of age Although the material is not complete it would appear that the diurnal variation first occurs between one and three months of age

Dr ANDERSSON (Stockholm) I shall report briefly some experimental and clinical results One hundred and ten rabbits were divided into three groups and were given respectively 12 25 and 50 mg of iron per kilogram of body weight intravenously in single doses They were killed from 1 hour up to 6 months after injection The internal organs were subjected to histological examination and the amount of hydrolyzable iron in liver spleen and bone marrow was determined

The most interesting findings were first that the total amount of hydrolyzable iron in the liver was directly proportional to the amount of iron given and amounted to about 40 per cent of the injected iron Secondly the iron in the liver decreased at a uniform rate of 0 13 mg per 24 hours This regular decrease could not be demonstrated in the spleen The histochemically demonstrable iron also decreased in proportion to the time elapsed from injection to killing The histological examination showed that the iron was first taken up by the reticuloendothelial system and after one to two weeks appeared in the liver cells

I could find no increased iron excretion in the urine but there were high values of iron in the bile from 5 to 14 days after the injection

The clinical investigation embraced 73 patients 52 women and 21 men all suffering from iron deficiency The total amount of iron injected per course of treatment was calculated to compensate for the hemoglobin deficit and over and above this an additional amount was given for storage in the iron depots

The hemoglobin concentration began to rise after 5 to 7 days The lower the initial hemoglobin value the higher the mean daily increase In 10 patients with hemoglobin values below 8 gm per cent initially there was a rise of 0 21 gm per cent per day

The serum iron value increased in all patients but did not reach normal levels unless more iron was administered than was required for hemoglobin synthesis On the other hand with adequate treatment the serum iron reached normal levels before the hemoglobin values had stabilized

Supernormal serum iron values were not observed The mean serum iron in the treated females was 116 μgm per cent and in a series of normal women it was 104 μgm per cent

Professor THEORELL (Stockholm) I think you injected the iron intravenously attached to a colloidal carrier whereas we injected it intraperitoneally as ferric ammonium citrate That is interesting because we expected to find an unusually high amount of iron in the liver since we injected it into the peritoneum but now it seems that the route of injection and even the form of the iron are immaterial In many experiments we arrived at the same figure namely about 40 per cent of the injected iron is found in the liver Apparently regardless of the manner in which iron is introduced into the body the same final distribution will be found at least in the liver It would be interesting to see if this is true with other organs as well

Professor VAHLQUIST (Uppsala) I think it is important to try to combine clinical experience with the highly scientific and very interesting information which has been given today I have one observation in connection with the lecture of Dr Laurell on transferrin

Perhaps it is not clearly realized by physicians that toxic signs will ensue if the amount of dialyzable iron injected intravenously is greater than about 0 15 mg per kilogram of body weight These signs are due to the fact that with such an amount of iron the saturation limit of the transferrin is surpassed The process of dialyzing this excess iron out of the blood stream seems to be extremely rapid In animal experiments with rabbits I have injected ferrous sulphate intravenously in amounts starting with 0 15 mg but increasing to

0 5 or even to 1 mg or 2 mg or iron per kilogram of body weight If one calculates the total amount of iron still present in the blood stream five minutes after the injection one finds that it is not more than about 25 or 30 per cent of that injected To me this indicates that there is an almost immediate equilibrium between the surplus iron in the plasma and the iron of other body fluids

Dr AGNER (Stockholm) Iron after its absorption from the intestinal tract is transported to different tissues where it can be used for the synthesis of different heme enzymes The cytochromes are relatively well characterized as to their function and structure In recent years Keilin and others have demonstrated that the catalases can be used in a system of coupled oxidation The physiological function of the peroxidases however is almost completely uninvestigated

Only two peroxidases have been isolated from mammalian tissues one from myelocytes and one from milk I am going to discuss myeloperoxidase, the enzyme isolated from the myeloid cells

This peroxidase is a protein with a molecular weight of about 200 000 to 250 000 It is a green iron porphyrin protein compound and has peroxidative activity It seems to be specific for the myelocytes and we have not been able to demonstrate it in any other tissue It constitutes 1 to 2 per cent of the myelocyte

Because of this specificity and the high content in myelocytes I became interested in whether this myeloperoxidase had any specific action in infectious diseases I tested several different systems in connection with this problem and found that the myeloperoxidase in the presence of hydrogen peroxide could detoxify diphtheria and tetanus toxins After such treatment the toxins could be injected into experimental animals in amounts corresponding to more than 100 lethal doses without any sign of toxic injury A crude toxin filtrate was used in the first experiments It was later found that the detoxification occurred only in the presence of some dialyzable oxidizable co-factor and that the same results could be obtained with purified toxin preparations only after adding a dialysate from the original crude toxin filtrate Addition of a previously oxidized co factor did not result in detoxification The mechanism for this process seems to be the following the dialyzable co-factor becomes oxidized by the peroxidase hydrogen peroxide complex and when oxidized reacts with and detoxifies the toxin

Dialyzable substances which can be used as co factors in the same way have also been found in human urine One of these substances has been identified as uric acid The toxin modified by some of the intermediary oxidation

products of uric acid does not however become completely detoxified The experimental animals develop paralysis to a large extent We do not know yet whether this is due to an incomplete detoxification or whether the original toxin has become modified in such a way that it becomes a special neurotropic toxin

These experiments have shown that diphtheria toxin can be detoxified by peroxidases in vitro It is possible that this peroxidative detoxification can occur also in vivo and this may be one of the ways in which the tissues respond to an infection

One more point In infectious diseases there is usually a decrease of the serum iron level At the same time there is an increase in the number of myeloid cells and thus an additional requirement of iron for the synthesis of the prosthetic group of the myeloperoxidase In order to make iron available for this synthesis and also for the synthesis of other iron-containing enzymes it may be necessary to administer iron in large amounts

Professor BESSEY (Chicago) I would like to make a comment and ask a question concerning the diurnal variation of plasma iron If the iron supply is a limiting factor from a nutritional point of view the diurnal fluctuation of plasma iron becomes much less pronounced than in the case of an adequate iron supply For instance if we determine at the same time each day plasma iron in adults given a constant adequate iron intake the plasma iron levels vary widely from say 80 to 175 μgm per 100 ml It seems that the factors involved in this variation are more complex than variations in intake However when dietary iron is a limiting factor if the plasma iron at the time we measure it is 60 μgm per 100 ml or lower it is consistently lower and stays in a fairly narrow range Has anyone studied the variations in subjects where the intake of iron was a limiting factor?

Professor VAHLQUIST (Uppsala) Concerning the regularity of the rhythm I quite agree with Professor Bessey that it is not ab olute from day to day If you follow the same person over several days you find various patterns just as you do if you follow the blood sugar on consecutive days When I made my first studies if I remember rightly of 18 people 15 showed a drop in serum iron from morning to afternoon and three showed a rise Thus there is no standard pattern of variation

The studies which Dr Agner is carrying out concerning new principles of the body s handling and neutralization of toxins by myeloperoxidase seem to me to be of fundamental importance It is very interesting to correlate these new observations with previously known facts concerning iron metabolism Dr Agner stressed the fact that during infection one finds serum iron values This is evidently due to a shift of the tissues which have priority in iron demand during infection

have considered these tissues to be chiefly the reticuloendothelium but evidently we have to reckon also with increased activity of the peroxidase system That a shift occurs is also evident from the fact that hemoglobin formation is impaired during infection

There is one point however on which I should like Dr Agner s opinion He ended by saying that in infection there is a low serum iron value and hence it might be useful to give iron There has been quite a lot of discussion about giving iron during infection For some time it was believed that one might thereby improve the anemia So far as I know this is not true With oral administration of iron one cannot influence the hemoglobin level as long as the infection continues It has been claimed and I have had some personal experience on this point that intravenous iron in large doses may increase the hemoglobin levels at least temporarily

On the other hand the serum iron level is a balance between inflow to and outflow from the blood and during infection there is a greatly increased outflow to the tissues One can prove this by performing an intravenous iron tolerance test So I am not certain that the low values really mean that there is a shortage of iron but I should like Dr Laurell s opinion To me they mean that there is a much more rapid outflow which cannot be balanced by inflow from the depots

Dr AGNER (Stockholm) We have not investigated this problem in detail We have had some experience with patients suffering from infections with periods of elevated temperature These patients had low values of serum iron The temperature became normal in a few days and they recovered rapidly after the administration of iron in large doses

Professor VAHLQUIST (Uppsala) Was that orally or intravenously administered iron?

Dr AGNER (Stockholm) Intravenously

Dr LAURELL (Lund) I must say that I quite agree with Professor Vahlquist that the outflow of iron from the plasma is so rapid that plasma iron values decrease during infection It is very interesting to hear from Dr Agner that treatment of patients suffering from anemia of infection with intravenous iron can rapidly restore their hemoglobin level to normal That has never before been pointed out in the literature

Dr AGNER (Stockholm) Dr Laurell must have misunderstood me I did not say anything about normalization of hemoglobin values after intravenous injection of iron in patients with infection I said that the temperature normalized and that the serum iron level may influence the iron-containing enzymes in different tissues more than the hemoglobin level I believe

also that it is more important to obtain a normal concentration of these *enzymes* than of the hemoglobin in these patients

Dr LAURELL (Lund) The pertinent question here is whether the low serum iron value is the limiting factor for synthesis of hemoglobin or of any other enzymes necessary to the defense mechanisms of the body The general experience with patients suffering from anemia of infection is that with fever the hemoglobin values fall rather rapidly and reach a steady state at about 50 per cent of the normal hemoglobin value If such patients can raise their hemoglobin levels during intravenous iron treatment before the fever has disappeared the beneficial effect of the intravenous iron will have been shown

Professor RÄIHÄ (Helsinki) Is anything known about serum iron in connection with vaccination?

Professor VAHLQUIST (Uppsala) We are just starting studies on iron metabolism in infants and small children during acute infections We also intend to follow not only serum iron but also the survival of erythrocytes during smallpox vaccination to see if there are any changes in these during the period of fever I do know that in animal experiments one finds a decline in serum iron if one injects toxins or if one produces a sterile abscess with turpentine

May I add one thing? Sometimes the influence of acute infection on the serum iron level may be very great There may be a competition I remember a patient with hepatitis which as a rule is accompanied by an increased serum iron level This child however had a simultaneous severe streptococcal adenitis It seemed that this infection was predominant so that there was a low serum iron in spite of the hepatitis

SURVEY OF COPPER METABOLISM IN CHILDHOOD

Dr AXTRUP (Kristianstad) Balance studies on animals and men have revealed that a small quantity of copper is retained in the organism The food consumed by the adult and by children weaned from a milk diet is fairly rich in copper

According to Macy's investigations (1944) a child between 8 and 11 years fed on ordinary food receives about 5 mg of copper daily The daily copper requirement for a child of the same age is about 0 10 mg per kilogram of body weight and as we know the weight of such a child is usually less than 50 kg Scoular found similar figures in his balance studies on boys between 3 and 6 years There is therefore no risk of copper deficiency in these ages

In infancy however the risk is present at least theoretically Prolonged feeding on milk might result in copper deficiency I shall come back to this problem later on

If the observations made by Sachs and co workers on dogs can be applied to humans food copper seems to be absorbed in the upper jejunal loop The element appears quickly in the plasma and continues to increase for 2 to 5 hours after which it falls abruptly The main excretion of copper takes place via the bile in the feces with only a small portion in the urine whose copper content is fairly constant and independent of the amount of copper in the food ingested

Small quantities of copper have been found in practically all organs and body fluids It has long been known that the copper content of the liver per unit weight is higher than that of other organs and that it is much higher in early life than in adulthood Prenatally there is no strict correlation be tween age or degree of development and copper content Even in very young fetuses the copper content of the liver is just as high as in full term subjects During the first year of life the liver copper falls steadily but is nevertheless still higher at the end of this time than in adults Between the ages of 1 and 5 the level is still slightly elevated and reaches adult level between 6 and 15 years Although some endocrine organs are also rich in copper the liver seems to be the chief organ of copper metabolism

Of greatest interest however is the blood copper The blood is known to contain about 110 μgm per 100 ml in adults the concentration in the serum and in the red blood cells being roughly equal Normally only slight varia tions are seen in blood copper and these are in the serum copper There are however some very interesting variations during prenatal life and just after delivery The serum copper value in fetuses 5 to 6 months old is high about 200 μgm per 100 ml and then gradually falls In the umbilical blood of the newborn it is very low about 50 μgm per 100 ml My own investigations in both premature and full term infants show that the copper curve rises from the low point at birth to a value of about 150 μgm per 100 ml during the first week It then falls to about 100 μgm per 100 ml both in premature and full term children During childhood it remains roughly at this level which is the same level as that of adults Serum copper is very high during preg nancy and at term it is more than twice as high as the normal value for healthy nonpregnant women and about five times higher than that of the infant

We know that copper is absorbed from the mother by the fetus But in what form? According to Holmberg s and Laurell s investigations blood copper is bound mainly to the globulin fraction Keilin and Mann have found a copper protein containing two atoms of copper per molecule called

hemocuprein Holmberg and Laurell have isolated another similar copper protein with eight atoms of copper per molecule which they call ceruloplasmin They suppose that ceruloplasmin contains four units of hemocuprein It seems reasonable to assume that the copper passes from the mother to the fetus as a copper protein for Holmberg and Laurell say that there is no free copper in the serum of the fetus or the mother All the serum copper seems to be present as a copper protein complex Thus if sodium diethyldithiocarbamate which forms a yellow complex with copper is added to serum this yellow color is not produced This indicates that the copper in serum is not free but is bound to a protein Furthermore serum seems to contain no free protein to bind more copper

How does serum copper behave in pathological conditions? In 1928 Krebs discovered that serum copper is high both in acute and in chronic infections and his results have been confirmed by a number of later investigations Heilmeyer found all infectious diseases to be attended by moderately or greatly elevated copper levels often twice the normal The increased copper content is usually accompanied by a somewhat elevated sedimentation rate After the infection subsided he found that the copper content and the sedimentation rate returned to normal Sometimes however the sedimentation rate was normal and the copper value increased in clear-cut cases of infection I have often seen this Copper may be a more sensitive criterion of infection than the sedimentation rate Heilmeyer has also shown by experiments on man that agents activating the reticuloendothelial system produce a rise in the serum copper Injections of milk for instance were regularly followed by a rise in serum coppe with a maximum one or two days after the temperature peak Injections of diphtheria toxin into horses had the same effect As soon as the antitoxin titer reached its peak the copper slowly returned to normal even if the toxin injections were repeated with larger doses When the antitoxin titer began to fall the copper once more responded to toxin injections as before This happens in the blood in infections

On the other hand the copper of the cerebrospinal fluid which is only one tenth that of the blood does not react in the same way in infection I have found that the spinal fluid copper is relatively constant even in cerebromeningeal infections

The experiences of the Wisconsin school in nutritional anemias in animals opened the question of the importance of copper in the formation of blood It was found that milk is poor in copper and it was therefore assumed that some of the infant anemias are due to a copper deficiency This view was borne out by a number of reports of successful therapy with copper either by

itself or in combination with iron in children suffering from anemia The clinical reports did not all agree however A number of authors were led by their own experiments to doubt the importance of copper in anemias of children Another circumstance suggesting that copper deficiency is not responsible for the anemias was that copper analyses of the blood in anemic children often revealed increased copper values Thus the data as to the importance of copper in hematopoiesis were contradictory

I wished to try to clear up this problem In view of the great tendency of premature children to exhibit deficiency diseases a study of prematures was thought to be especially useful to determine whether copper deficiency can arise It is true that the copper content of the liver of premature children is not lower per unit weight than that of full term children But as the gross weight of the liver is less in prematures its total copper is also less The series I studied therefore consisted largely of premature children and twins who may be regarded in many respects as prematures A number of other anemic children were also examined

The premature and twin series 120 children in all were followed with regular determinations from shortly after birth to about 6 months of age *In these series the copper values were regularly about 107 μgm per 100 ml* although quite severe anemia had developed Premature children showed no difference from full term children all having the same value for this first half year of life Nevertheless some of the infants were treated orally with copper in the form of copper sulphate No permanent rise in the *copper level was obtained Nor had the copper treatment any noticeable* effect on the hemoglobin or the red blood cells despite prolonged and intensive therapy When iron treatment was commenced after the copper treatment there was a rapid rise of hemoglobin and numbers of red blood cells after a short period In order to find out whether copper stimulated iron in its action 9 pairs of twins were treated with copper and iron in such a way that one twin of a pair was given copper during a period while the other one received no treatment Both of them were then treated with iron No difference could be seen the iron treated twin reacted just as quickly as the copper and iron-treated twin

Besides premature children a study was also made of a number of anemic children aged 6 months to 2 years Several of them could doubtless be classed as cases of nutritional anemia But the blood copper values of these children were roughly the same as in full term nonanemic children Copper treatment gave no permanent rise of the blood copper level However the blood copper level reacted at once in infections another indication that the copper supply of *the body was not exhausted*

In rare cases administered copper seems able to stimulate hematopoiesis even when the intrinsic copper supply in the organism appears sufficient When this last series was treated with copper and iron it was possible to register an effect of copper treatment in a couple of cases where the copper was given after the iron treatment had commenced These were 2 patients with very protracted anemia who reacted very badly to iron treatment When copper was begun there was a distinct and fairly prolonged rise of reticulocytes though only reflected in a very slow and slight improvement of the anemia Usually however as we all know these anemias react excellently to iron therapy There should therefore be hardly any reason to make a special addition of copper to the iron preparations

As a general conclusion then it can be stated that there is no need to fear a copper deficiency in infants They have sufficient reserves to cover their copper requirements The blood copper seems to be governed by endogenous factors and apparently remains constant no matter whether the individual is suffering from anemia or not but is very labile in the pre ence of infection

The role of copper in hematopoiesis is still obscure It is not a cons ituent of the hemoglobin molecule It seems to act in some way upon red blood cells but we do not know how

Finally there are several other recognized functions of copper which show the great importance of this metal Copper may take part in the production of melanin DOPA is oxidized by copper There may therefore be some connection between the very high serum copper and the pigmentation of the skin in pregnancy The endocrine organs are rich in copper Does that imply another function of the metal? This and other important copper problems still await further research

STUDIES ON SERUM COPPER

Dr LAURELL (Lund) Abderhalden showed in 1928 that serum copper is nondialyzable The copper becomes dialyzable however if serum is acidified In 1939 Mann and Keilin prepared a crystalline blue copper protein from blood corpuscles and horse serum This protein was named hemocuprein As they suspected that hemocuprein might have some catalytic activity like other known copper proteins they tested it against various substances but could find no substrate If hemocuprein was treated with strong reducing substances it lost its blue color permanently The molecular weight of their copper protein was astonishingly low 35 000 when we consider that practically no copper is found in the urine Molecules of these

dimensions usually pass into the urine but human urine is practically copper free

Dr Holmberg and I later worked out a method to isolate the native copper protein from plasma This protein has a fairly strong blue color and has therefore been named ceruloplasmin Spectrophotometric investigation has shown that even the native serum copper complex is blue The color of serum from pregnant women is yellow green as compared to normal yellow serum and the same is true of sera from patients with severe acute or chronic infections Sera from such patients have as we have heard a high copper content that is a high concentration of ceruloplasmin

Ceruloplasmin differs from hemocuprein in some important respects Ceruloplasmin seems to contain four units of hemocuprein as their molecular weights are about 150 000 and 35 000 respectively In contrast to hemocuprein ceruloplasmin can also be reversibly reduced and oxidized When reduced it can be reoxidized by atmospheric oxygen The reduced form is colorless in contrast to the blue oxidized form We have not as yet succeeded in showing whether ceruloplasmin takes up oxygen as the hemocyanins do or if the color change occurring upon treatment with reducing substances depends upon an electron increase in the chromophoric group If ceruloplasmin can bind oxygen this bond must be extremely firm as not even lyophilizing decolorizes it

As the reduction of ceruloplasmin is completely reversible in the presence of oxygen it was suspected that ceruloplasmin could serve as an oxidizing enzyme It was shown that ceruloplasmin can act as an oxidase Its enzymatic properties resembled those of laccases which had earlier been isolated from plants Just as in the case of laccases paraphenylenediamine (PPD) was the substrate found which was most easily oxidized by ceruloplasmin The activity of the enzyme is highly dependent upon the concentration of the inorganic ions present and upon pH Some polyvalent ions such as phosphate and citrate lessened the activity of the enzyme and some monovalent ions such as chloride and nitrate increased the activity At neutral pH the catalytic activity is small and optimal activities are obtained in the pH range between 5 and 6 Substances of biological and clinical interest which have been found to be oxidized by ceruloplasmin are epinephrine and ascorbic acid

If it is assumed that ceruloplasmin is the only substance in serum which catalyzes the oxidation of PPD and if it is also assumed that nearly all the copper in serum occurs as ceruloplasmin there ought to be a direct proportionality between the velocity with which serum catalyzes the oxidation of PPD and the copper content of serum We have tested the velocity with which about 20 different sera oxidize PPD These sera were collected from

umbilical cord blood from normal individuals from pregnant women and from patients suffering from infection and leucemia This selection was made to obtain the greatest possible variations in serum copper The results showed that there is a direct proportionality between the copper content of serum and the oxidation velocity of PPD These results indicate that practically all the copper in serum is bound in ceruloplasmin molecules

Since the beginning of this century papers have occasionally appeared in which observations have been pres nted to elucidate the occurrence of oxidases and peroxidases in human plasma Nobody tried to isolate any oxidase or peroxidase from human plasma but it had been shown that adrenaline and DOPA are oxidized more rapidly by sera from pregnant women than by sera from umbilical blood These facts now seem comprehensible since ceruloplasmin oxidizes these substances and the concentration of ceruloplasmin is about five times as high in pregnancy sera as in fetal sera

Henning and collaborators Mollerstrom and others are of the opinion that peroxidases exist in human plasma They have been able to show that different sera oxidize benzidine with different velocities in the presence of hydrogen peroxide The hypothesis has been advanced by Agner that the peroxidative reaction which has been observed in human serum may depend upon the existence of verdoperoxidase in different concentrations in serum He believes this verdoperoxidase is derived from disintegrating leucocytes We have however, been able to show that benzidine is oxidized readily by ceruloplasmin and by serum in the presence or absence of added hydrogen peroxide There is furthermore a direct proportionality between the velocity with which benzidine is oxidized and the copper content of the serum used This means that the main benzidine-oxidizing sub tance in erum is ceruloplasmin and not verdoperoxidase If any verdoperoxidase exists in normal or pathological sera its concentration must be extremely low as even sera from patients with myeloid leucemia do not oxidize benzidine more rapidly than can be expected from the copper content of these sera

CHAIRMAN Perhaps we could now proceed to the introductory talks on zinc metabolism and then go on to the discussion of copper and zinc metabolism

SURVEY OF ZINC METABOLISM IN INFANCY

Dr BERFENSTAM (Uppsala) The first scientific reports concerning the distribution of zinc in the organism can be found in the literature of the 1850 s After this it became apparent that zinc was present in foods

from vegetable as well as animal origin and was also found in most organs in the body It was not until relatively recently however that questions as to the biological importance of this metal arose and only after it became known in 1920–30 that zinc was a necessary constituent of certain enzymes and hormones was its importance appreciated For example the enzyme carbonic anhydrase which catalyzes the breakdown of carbonic acid to carbon dioxide and water and is essential for normal carbonic acid metabolism is a zinc protein compound It is present in most organs and especially in red blood cells

It has been calculated that adult humans contain about 2 gm of zinc The daily intake is about 10 to 15 mg which is about the same as that of iron The presence of varying amounts of zinc can be demonstrated in all organs and also in milk sweat urine and feces The zinc content of the plasma is about the same as that of iron and copper whereas the zinc content of the white blood cells is very high Red blood cells contain about 1300 μgm per cent this being very much higher than the corresponding value for copper but of course much lower than the iron content

My interest in zinc investigations started with certain enzyme studies in children I was able to verify the finding of Stevenson that the carbonic anhydrase activity in newborn and especially in premature children is very low in comparison with the activity of the enzyme in adults According to my results the values for premature infants were even lower than those found by Stevenson and amounted to only 5 to 15 per cent of the normal value for adults The activity is proportional to the degree of development that is underweight but otherwise almost fully developed babies show a higher activity than premature children with a relatively high weight

Whether or not the low carbonic anhydrase activity really has any bearing upon the disposition of premature children toward cyanosis as Stevenson suspected is difficult to say Transfusion of adult blood into premature infants raises the carbonic anhydrase activity besides having a favorable effect on the general condition of the child But of course so many factors are influenced by a transfusion that it would be difficult to ascribe the general improvement to the increase in this particular enzyme activity I have studied this increase in activity and found that it cannot be accounted for solely by the contribution of the injected blood cells It would appear then that the plasma from adults has a direct activating effect on the enzyme of the premature infant

Having these enzyme investigations in mind work was begun on the zinc content of plasma and erythrocytes It seemed of interest to investigate the

possible low zinc content in red blood cells of premature infants (corresponding to the low carbonic anhydrase activity) the behavior of zinc in mother and child upon parturition and the transport of the metal through the placenta

The literature gives scanty and contradictory information about the normal zinc content in the blood of adult humans That is probably due to the fact that the methods for determination of zinc were unsatisfactory During recent years more reliable methods have however been developed In one of these developed by us the protein zinc bond is destroyed by treatment with strong hydrochloric acid and the free zinc is determined with dithizone The method is accurate and has the advantage of being so sensitive that even the small amounts of blood obtainable from the capillaries are sufficient for analysis Using this method the normal value for healthy adults was found to lie in the neighborhood of 110 μgm per cent for plasma and 1300 μgm per cent for red blood cells

It was also shown that the zinc content in the blood corpuscles of newborn children was low being about one third that of adults The premature infants showed even lower values In the first year of life a sharp increase in the zinc content takes place but the adult level is in general not attained until adolescence If one compares the carbonic anhydrase activity and the zinc content of red blood corpuscles a good correlation between these quantities can be found Even though it would not be safe to draw the conclusion that all the zinc in the blood cells is bound to this enzyme the evidence strongly indicates this

In order to study the transport of zinc investigations were extended to the zinc of serum which has no carbonic anhydrase activity The zinc content of serum is of the same order of magnitude as that of copper and iron and constitutes probably the transport zinc It is rather constant in healthy persons not only during different times of the day but also in samples taken from the same individual at intervals of several months

One condition in which variations occur is in pregnancy in which the concentration of zinc in the serum decreases At parturition therefore a peculiar situation prevails with the zinc content of the plasma of the mother lower than that of the plasma of the infant The same variations are found in iron distribution during pregnancy and parturition but the behavior of copper is the exact opposite inasmuch as this metal increases during pregnancy but is very low in the newborn child

Newborn and especially premature children have as we have seen a low zinc content in their red blood corpuscles associated with a low carbonic

anhydrase activity It is possible that a higher enzyme concentration could be of value to an organism which like that of the premature infant is unde veloped in several respects such as the *insufficient general circulation with its* coarse capillary network and the incompletely developed respiratory center

An obvious step would then be to try to increase the zinc content of the red corpuscles by supplying zinc to the blood In order to achieve this it might be worthwhile to try to influence the zinc content of the newborn by treating the mother during pregnancy Besides it would be interesting to determine whether the zinc content of red cells could be made to increase more sharply than it already does during the first weeks of life with additional zinc Furthermore it would be desirable to determine the response of adults to increased amounts of this metal

Such experiments naturally cannot often be made on humans directly but since it could be shown that newly born rabbits had a considerably lower zinc content in their red blood cells than adult rabbits—thus showing an *analogy to humans—the tests were performed on these animals*

The purpose of the following experiments was then to study the effect of a large zinc supply on (1) adult rabbits (2) pregnant rabbits and (3) rabbits during their first months of life

Rabbits were given zinc salts orally as well as parenterally The oral treat ment resulted in a marked and lasting increase of the zinc content of serum and was therefore used exclusively in the later experiments Working with the necessary controls it could be established that the plasma zinc content could be kept at a high almost constant level through a daily intake of zinc salt

The next step then was to investigate whether the red blood corpuscles of adult rabbits were capable of taking up extra zinc from the zinc-enriched plasma The result was however that in spite of keeping the zinc content grossly elevated for a period of months it was not possible to produce a persistent increase in the concentration of zinc in the cells

In order to test whether the red blood cells of newly born rabbits were more susceptible to an increased zinc supply zinc salts were fed to pregnant rabbits In spite of the high zinc content in the mother s plasma the zinc content in the red blood cells of the offspring remained as low as that of the controls

Experiments designed to influence the normal increase of zinc in young rabbits *were also performed No special zinc feeding is necessary* since the milk of the zinc fed mothers was very high in this metal so that the young automatically received a high dose of zinc in their natural food This how ever also caused only the plasma zinc content to increase with only a transient effect on the zinc in the erythrocytes

I have had some occasion to study the influence of increased zinc supply to newborn children These were all cases of infants incapable of living because of malformation of the central nervous system The zinc was given orally in the form of a zinc salt solution mixed with milk During the experiment the children got 50 mg of zinc acetate a day which is about fifty times the amount normally present in food but no variation of the zinc content of the red blood cells could be observed

It appears therefore that the zinc content of the red blood cells cannot be influenced at least by the methods used so far

STUDIES ON SERUM ZINC

Dr VIKBLADH (Lund) With two quite different quantitative methods I have been able to show that zinc is present in serum in at least two states one firmly bound and one more loosely bound fraction Normal individuals have according to my investigations a total zinc content in serum of 125 μgm per cent One third of this that is about 40 μgm per cent belongs to the firmly bound fraction while the rest is more loosely bound Sera from patients with different diseases rarely show increased zinc values but the zinc content is decreased in many conditions This decrease is in the loosely bound fraction

In patients with acute fever—for instance pneumonia—one can see this fact very clearly The total zinc content may be decreased to about 50 to 60 μgm per cent During recovery the zinc content will go up to about twice this amount that is to the normal value The firmly bound fraction however always remains practically unaltered This means that the loosely bound fraction varies from practically zero to about 80 μgm per cent

I suppose therefore that the loosely bound fraction is responsible for the transportation of zinc in the body The firmly bound fraction on the other hand probably has another function

Not only are serum zinc values decreased in patients with fever but patients with chronic infection and severe disease generally exhibit similar findings Thus one finds low values in patients suffering from chronic polyarthritis hepatic cirrhosis ulcerative colitis chronic nephritis and so on In nephrosis too the zinc content in serum is low This is similar to what has been found for serum copper and probably due to a loss of zinc-combining protein through the kidneys

Diseases of the blood are the most interesting with respect to zinc variations In leucemia I have found that the serum zinc is considerably lower than normal I have not seen any definite changes during therapy

According to Vallee and Gibson the zinc content of the erythrocytes is significantly elevated in pernicious anemia This is not solely due to an increase in cell size Under successful liver therapy there is a progressive fall in the zinc content of the erythrocytes which reaches normal values when the red blood cell count rises to normal

I have found the serum zinc to vary in the opposite direction from the red cell zinc in pernicious anemia Thus in untreated patients the serum zinc content is low The value begins to rise on the second day after institution of liver therapy By the fourth to seventh day the serum zinc value is normal

This variation in serum zinc thus constitutes a mirror image of the variation found in serum iron when pernicious anemia is treated Theoretically this might be explained according to the hypothesis of Cohn and collaborators by assuming a competition between iron and zinc for the same protein in serum It should be added here that in the anemic patients with low serum iron whom I have studied the serum zinc was not increased

Experiments performed in which the serum iron level was increased to near the saturation limit either by intravenous injection or by oral ingestion of iron have yielded results which are difficult to interpret The diurnal variations in serum zinc must be taken into consideration when the results of these experiments are discussed

In my normal subjects the serum zinc level seems to reach a maximum in the early morning and a minimum at 3 to 4 o clock in the afternoon The magnitude of the variation is fairly great with mean values of 123 μgm per cent at 9 A M and 113 μgm per cent 6 hours later

When iron was injected intravenously into normal subjects I got at corresponding times a decrease in serum zinc from 123 μgm per cent to 81 μgm per cent The largest fall occurred during the first half hour

When iron was given by mouth identical results were obtained But if iron was taken at 3 A M the serum zinc was practically unaltered at 9 A M even though the serum iron was high At 3 P M however the zinc values were lower than in the control subjects The fact that the zinc content is not at its lowest when the iron has reached its maximum may possibly be explained by a slow zinc elimination rate

After intravenous injection of zinc into healthy persons I have found that normal zinc values are not reached until six hours after injection and that there is no influence upon the serum iron

It is therefore not yet clear what the explanation is for the variations in zinc and iron in pernicious anemia

Professor THEORELL (Stockholm) Dr Axtrup has drawn the conclu

sion that copper must pass from mother to fetus combined with the protein component ceruloplasmin with a molecular weight of about 150 000 That is a rather large molecule to pass through the membrane of the placenta is it not? You also mentioned that the addition of diethyldithiocarbamate did not show any color shift Does that mean that no copper is liberated from cerulo plasmin by diethyldithiocarbamate?

Dr AXTRUP (Kristianstad) To answer your second question first this experiment shows only of course that the link between copper and pro tein in ceruloplasmin is firmer than the link between copper and diethyl dithiocarbamate

With regard to your first point it is not necessary to assume that the protein copper molecule passes the placenta as such The result i the same if the copper passes as an ion and is taken up immediately by the protein on the fetal side

Dr AGNER (Stockholm) I would like to ask Dr Laurell what hydrogen peroxide concentration he used in his experiments on oxidation

Dr LAURELL (Lund) I performed these experiments in exactly the same way as those who concluded that there was peroxidase in plasma I wished to see if the activity observed by them depended upon peroxidase or upon ceruloplasmin They diluted three drops of concentrated hydrogen peroxide to 100 *ml* with water and they used two drops of this solution in a 5 *ml* volume for their tests The final concentration of hydrogen peroxide is not very small and I know that Dr Agner has shown that the concentra tion of hydrogen peroxide should be extremely small to get the best activity of peroxidase What I want to point out however is only that the data in the literature concerning variation in peroxidase concentration in blood are based on experiments which have not shown that there is any peroxidase at all in blood! At least this is true of the experiments of Mollerstrom and of Henning and collaborators Some French authors have also reported ex periments which Professor Theorell knows of about peroxidase in blood I am almost sure that their investigations were performed on whole blood and not on serum and so I have not compared their results with ours

Professor THEORELL (Stockholm) They presented the rather peculiar hypothesis that hemoglobin is the most important of all peroxidases in the body which I do not believe at all But there ought to be a special plasma peroxidase

Dr AGNER (Stockholm) I agree with Dr Laurell that earlier published experiments done in order to demonstrate the existence of a peroxidase in serum do not fulfill present requirements since we have found out some

the properties of myeloperoxidase Myeloperoxidase is very sensitive to hydrogen peroxide is inhibited at concentrations above 10^{-4} molar and destroyed above 10^{-3} molar If we try to use common tests for peroxidase and add hydrogen peroxide to a concentration of about 10^{-4} molar we have to take into consideration that catalase decomposes hydrogen peroxide of that concentration very rapidly We have tried to study this problem by using other techniques but have found it very difficult At the present time there are no conclusive proofs for the existence of a peroxidase in plasma It seems very reasonable that there may be one but other methods will have to be worked out before we can demonstrate it for certain

Professor VAHLQUIST (Uppsala) Professor Theorell raised the interesting question of the form in which copper passes from mother to fetus I agree with him that it is difficult to judge on the basis of facts now available whether it is in the ionized form or combined with protein On the other hand coming back to the question of placental antibody transfer we know that high molecular weight proteins can pass through the placenta Antitoxins which have molecular weights of around 170 000 do this and the isoagglutinins which as far as I know have even higher molecular weights do also

There are two other membranes which are interesting in this connection We have heard that the copper content of the cerebrospinal fluid is low and that it is not appreciably influenced by inflammatory conditions Nothing has been said about iron Iron is present in the cerebrospinal fluid too at a concentration of about 30 or 40 μgm per cent The iron in the cerebrospinal fluid however changes materially with inflammatory conditions showing a decrease in meningitis for example

From Dr Berfenstam we heard that oral administration of zinc to a lactating animal increases the zinc content of the milk That seems to be the opposite of the situation with iron Such experiments have been done among others by Dr Wallgren with oral ingestion of iron If I remember correctly there was no effect on the iron content of the milk It might be interesting to find out whether it would be possible to increase the iron content of milk by *intravenous* injection of iron

Professor LINDERSTRÖM LANG (Copenhagen) I should like to call attention to the fact that zinc has been shown to activate peptidases I do not know what relation this has to carbonic anhydrase but it is quite possible that the amino groups of proteins and peptides in the body exist to some extent as carbamates The activation of peptidase by zinc can be inhibited by cyanide

I should also like to call attention to the high concentration of peptidase in red blood corpuscles a fact which is not generally known This was found by Johansen and Thygasen It is an aminopeptidase that will split substrates like alanylglycylglycine It can be prepared from red blood corpuscles by removing hemoglobin and by filter paper chromotography The purified enzyme is very stable in neutral solution but decomposes rapidly below pH 4

CHAPTER VII

Panel on Isotopes in Metabolism

ISOTOPES IN MEDICINE

Professor de HEVESY (Stockholm) The application of radioactive substances in medical therapy aims at the replacement of external irradiation by X rays or other penetrating radiation by irradiation in situ by beta rays and gamma rays emitted by radioactive substances taken up by the tissue

In view of the fact that most radioactive elements are distributed in all organs one would expect that those few elements which concentrate in one or a few organs would be used for therapeutic purposes To some extent this is the case Iodine which is representative of the type of element which concentrates in one organ has found wide application and Dr Barnett will tell us more of this later

Radiophosphorus is distributed to all organs more or less equally although in greater amount to growing tissue and with a strong tendency to accumulate in the skeleton In spite of the fact that the bone marrow does not concentrate radiophosphate selectively sufficient local irradiation is produced following the administration of a few millicuries of P^{32} to interfere with mitotic processes and thus with the formation of red and white corpuscles P^{32} is used with great success to suppress an overabundant formation of red corpuscles in polycythemia We meet here an example of successful application of radioactive compounds in medical therapy In view of the fact that at present radioactive isotopes of almost every element are available the future may bring many other succesful applications in therapy

Radioactive isotopes have however found their main application in the field of medical diagnosis and in the related fields of physiology and biochemistry I want to discuss a few of these applications

Almost immediately after the discovery of heavy water efforts were made to determine how long orally ingested water molecules remain in the body

In these experiments water labeled by the presence of heavy water was ingested and the heavy water content of urine collected at different times was determined A few minutes after the intake of such water some heavy water could be found in the urine A small though easily measurable fraction was excreted through the kidneys before exchange equilibrium between intra and extravascular water took place By an hour or so later the ingested water was in exchange equilibrium with the total body water and the labeled water was being excreted according to an exponential relation The water molecules were found to have a mean lifetime in one experiment of about a fortnight The exact figure obtained for the lifetime of water molecules in the body depends on the amount of water taken in and may differ in winter and summer The figure mentioned above was obtained on a hot summer day when the human subject perspired a great deal thus shortening the life cycle of water molecules in his body As the labeled water is excreted according to an exponential relation we can easily calculate the time needed for disappearance of a certain fraction of labeled molecules By doing so we arrive at the result that in spite of the fact that the body contains an almost astronomical number something like 10 of water molecules a child of two does not contain more than one or two water molecules of those it received from its mother at birth

In the experiment just described the total water content of the organism can be determined as well If for example 10 ml of heavy water are administered and after an hour or two when exchange equilibrium has been attained we find in 1 ml of plasma water a heavy water concentration of 1 1000 then we can conclude that 10 000 ml of body water participated in the dilution of the heavy water or that the body water content amounts to 10 liters This simple calculation neglects the fact that about 3 per cent of the administered deuterium undergoes exchange with organic molecules The results of this convenient method depend on the obesity of the person investigated Quite recently I saw the results of a very extensive investigation utilizing heavy water as an indicator in which it was shown that the body water of obese persons was 44 per cent of body weight while in lean individuals 77 per cent of the body weight is water

The recent availability of radioactive (tritium) water makes it possible to replace heavy water by superheavy water in such experiments While the heavy water content of body fluids is assayed by density or mass-spectrographic measurements the superheavy water content is assayed by radioactive measurements which are more convenient and more sensitive Such investigation can be carried out by exposing the human subject to a total radiation dose of not more than 0 14 r e p Studies on total body water content

using superheavy water have been carried out at the Donner Laboratory These investigations have also shown that the total body water content varies greatly from individual to individual the variations reflecting for the most part variation in body fat

In a similar way using labeled sodium chloride we can measure the life time of sodium ions It is even possible to calculate the lifetime of water molecules or that of sodium ions in the body without making use of isotopic indicators The knowledge of total water content of the body of the amount of water taken in daily and of that formed by catabolic processes suffices for the calculation But prior to the application of isotopic indicators to such determinations no one thought of these possibilities This emphasizes that isotopic indicators have drawn our attention to certain lines of thought which were previously neglected It took only a year or two before this notion was applied by Schonheimer and Rittenberg in their famous work on the renewal rate in various organs of fatty acids proteins and other compounds For example they showed that the half life of the average fatty acid molecule of the liver amounts to about 2 days and that the corresponding value for the fatty acids of the carcass is much longer

When carrying out experiments with isotopic indicators we can follow two lines of experimental attack One is to keep the indicator in the body fluids at a constant level Schonheimer and Rittenberg for example let their rats drink water containing 3 4 or 5 per cent heavy water for weeks or months and brought the heavy water content of the rat to a constant value After a fortnight they found the carcass fatty acid hydrogen contained half as much deuterium as did the body water hydrogen from which they concluded that half of the carcass fatty acid molecules had been newly formed during the experiment Another method of attack is to administer the radioactive indicator for example radiophosphorus or radiocarbon at the start of the experiment This will rapidly disappear from the circulation This decrease of the activity of the blood plasma with time has disadvantages since it complicates the calculation of the results but it also offers great advantages

We have long had nonisotopic methods for determining the percentage of body water which is extracellular Any compound which does not penetrate into cells can be applied in such determinations Thiocyanate is one example A few hours after injecting a known volume of a thiocyanate solution we determine the thiocyanate concentration in plasma water and calculate from the dilution of the injected thiocyanate the water volume which participated in this process and which is supposed to be identical with the extracellular water volume

The application of radioactive isotopes makes it possible to label some of

the physiologic plasma constituents We can label sodium by adding radio-sodium or chloride by adding radiochloride The radioactivity of such samples is minute and the radiation emitted by them can do no harm Radio-sodium and radiochloride have found extensive application both in the determination of the extracellular volume of the total body nd also that of single organs

After injecting labeled sodium chloride into the circulation of a rabbit we took plasma samples at intervals and measured their radioactivity Figure 128

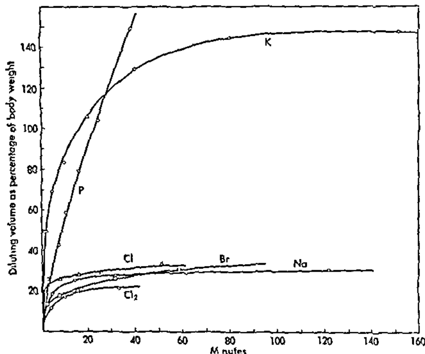


Fig 128 Rate of disappearance of various labeled ions from the plasma

gives the results The abscissa indicates the time in minutes and the ordinate values do not provide corresponding radioactivities but indicate the water volumes which are necessary to dilute the radioactive sodium to the same concentration it has in the plasma The disappearance of Na from the plasma is very rapid After one minute about one half of the radioactive sodium has disappeared Now radioactive sodium behaves in the same way as does common sodium present in the plasma Thus half of the sodium ions which were present in circulation at the start of the experiment were no

longer present after the lapse of a minute and were replaced by sodium ions formerly in the tissues The latter moved into the plasma while sodium in the plasma moved into the tissues Figure 128 also shows clearly the difference between elements like sodium chloride and bromide which remain chiefly in the extracellular fluid and elements like potassium and phosphorus which are chiefly intracellular The latter penetrate into cells and this being a slower process takes much longer than the passage through the capillary wall In

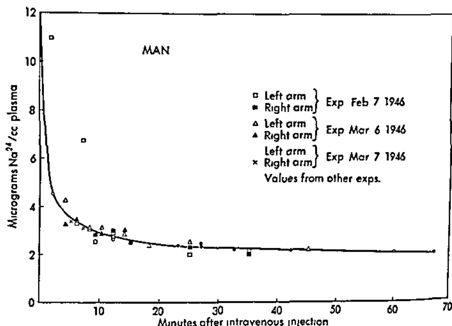


Fig 129 Plasma concentrations of Na following intravenous injection (Flexner L B Cowie D B and Vosburgh G J *Cold Spring Harbor Symp Quant Biol* 13 92 1948)

the case of sodium chloride exchange equilibrium is obtained in the rabbit in the course of 20 minutes The extracellular volume of the rabbit is calculated to be about 23 per cent of body weight Similar figures are found for the extracellular water content of human subjects In infants however much higher figures are found Flexner and associates give values up to 45 per cent of body weight for the extracellular space of infants and for that of the fetus as much as 65 per cent

Figures for the rate of disappearance of Na^{24} injected into the human circulation are seen in Figure 129 as obtained by Flexner and associates The rapid disappearance of labeled sodium from the circulation is at first sight very puzzling But it is no longer strange in the light of the following

fact the amount of material which diffuses is always proportional to the surface through which diffusion takes place One milliliter of plasma is in contact with not less than 6000 sq cm of capillary wall as Krogh has shown With such a tremendous surface through which sodium ions can pass it is no wonder that they leave the plasma with very great rapidity to be replaced by sodium ions from the tissues moving in the opposite direction It follows from the above figures that in the human about 4 gm of sodium are ex changed between the circulation and the tissues every minute

In the experiments described above plasma samples are secured placed

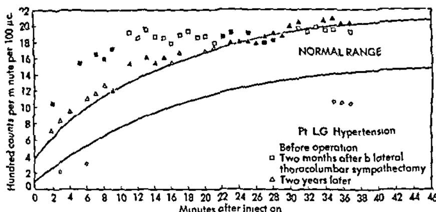


Fig 130 Radiosodium build up curves in a patient with severe hypertension relieved by thoracolumbar sympathectomy Black symbols refer to the right leg white symbols to the left leg (Quimby E H Radioactive Sodium as Tool in Medical Research *Am J Roentgenol* 58 746 1947 Charles C Thomas Publisher)

under the window of the Geiger counter and their β activity measured But sodium also emits very hard penetrating gamma rays which can be measured outside the body In experiments of Dr Quimby in New York the gamma rays of sodium are used Sodium chloride is injected into the arm a Geiger counter is applied to the foot and the time taken for the injected sodium to reach the foot is measured It is found to be 20 to 55 seconds In circula tory disturbances the time is correspondingly longer Instead of a single ve locity of propagation a so-called build up curve can be made by measuring the gamma ray activity over the foot at different times In pathological states the trend of the build up curve differs from that observed for normal individuals and the effects of medical or surgical therapy can be studied by changes in propagation velocity or build up curves Figure 130 shows build up curves before and after sympathectomy in a 40-year-old man with a severe circulatory disturbance in his legs

Radiosodium (Na^{24}) is a very suitable indicator for circulation velocity measurements although for other purposes Na with a half life of two years may be more useful That the half life of Na^{24} is only 14 hours is an advantage since radioactivity does not remain in the patient long and the danger of possible radiation damage is thus minimized Short term activity is sufficient to carry out such measurements

Another experiment of Dr Quimby evaluated the effectiveness of artificial respiration in moving blood Half an hour after death a small quantity of radiosodium was injected into the femoral artery or vein of a dog whose blood had been heparinized The counter was placed over the carotid jugular region and the resuscitator started The results are seen in Figure 131

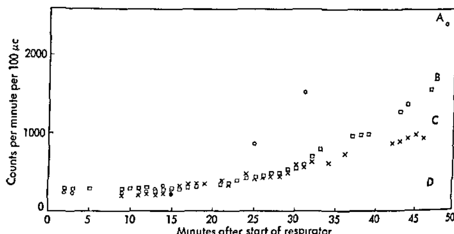


Fig 131 Radiosodium build up curves demonstrating movement of blood by artificial respiration in dead but heparinized dogs *A* respirator using alternate pressure and suction *B* suction alone *C* pressure alone *D* no respirator (Quimby E H Radioactive Sodium as Tool in Medical Research *Am J Roentgenol* 58 749 1947 Charles C Thomas Publisher)

After a period of artificial respiration radiosodium arrived in the vessels under the counter and continued to increase in amount from 30 minutes to an hour *In control animals with no respirator or heparin the material* was never demonstrated above the diaphragm

A difference between the technique of keeping the isotope activity level constant during the experiment and the administration of the radioactive indicator only at the start of the experiment may be seen in the disadvantages of the latter method when applied to the problem of the renewal of the mineral constituents of the skeleton These were actually the first studies carried out with artificial radioactive elements as indicators

If one administers labeled phosphate an appreciable part of this P is soon found on the surface of the apatite like crystals of bone Then for a while not much happens since it takes some time until the underlying molecular layers of bone crystals are involved in a renewal process We must envisage the mechanisms involved at times after meals for example phosphate calcium magnesium glucose and phosphatase concentrations are enhanced and plasma is supersaturated with respect to the mineral components of bone As a result molecular layers containing P are formed on the surface of the apatite like crystals On the other hand at those times when the concentration of the formative elements of bone in the plasma is low the opposite phenomenon occurs However it is also quite possible as emphasized recently by Neuman that some recrystallization of bone takes place without fluctuations in the concentration of plasma constituents Minerals which are not properly crystallized can spontaneously recrystallize

Thus at the start of the experiment when the P content of the plasma phosphate is high molecular layers will be laid down which are highly active With time most of the plasma enters the tissues or is excreted the plasma activity thus declines rapidly with the result that molecular layers of much less activity are laid down possibly on top of the highly active ones which are now enveloped The probability of release of the trapped P is very much diminished In a similar way following lead poisoning some lead is trapped in the mineral constituents of the skeleton During the early stages of lead poisoning the plasma contains a high level of lead and layers in which calcium is partly replaced by lead are formed Later as most of the lead disappears from the circulation molecular layers containing only very little lead may be deposited above the lead rich layers which thus are trapped and may remain in the skeleton to become a source of chronic lead poisoning Aub who carried out extensive studies on lead poisoning treated his patients with large amounts of ammonium chloride to increase the acidity of the blood in order to dissolve the uppermost molecular layers and release the trapped lead

Because of the complicated mathematical function which describes the way in which the plasma activity decreases and the molecular layers are laid down in the skeleton it is hardly possible to calculate from the specific activity of plasma and bone phosphate the rate of renewal of the skeleton If however we inject labeled phosphate several times daily all through the experiment in order to keep the radioactivity level of the plasma phosphate constant then we can easily calculate the extent of renewal of the mineral constituents of the bone

We inject P' for two months for example and then compare the radio-

activity of 1 mg of plasma inorganic phosphorus and of 1 mg of bone phosphorus We can state at once then to what extent the mineral constituents of bone were renewed If they had been fully renewed, we would get the same figure for the activity of 1 mg of bone phosphorus as for 1 mg of plasma phosphorus *This however is not the case As you see from* Figure 132 in diaphyseal tissue only about 6 per cent of the bone phos

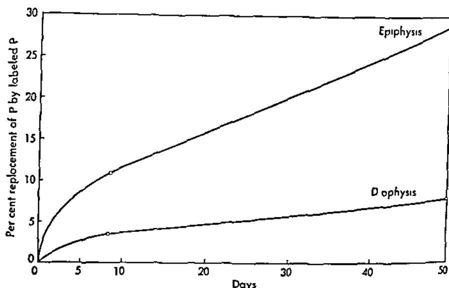


Fig 132 Extent of repla ement of rabbit s bone phosphorus by labeled phosphorus

phorus was replaced The renewal of the mineral constituents of soft bone is more pronounced as 26 per cent of the mineral constituents of the epiphysis were renewed in the same time These are mean figures obtained for di aphyseal and epiphyseal samples of the bone tissue Different sections of these samples may show a strongly differing P^{32} content

No such experiments have been carried out on human subjects but it seems reasonable to conclude from the above figures that in the adult human body a very large part of diaphyseal tissue remains unchanged during life It must be pointed out that time does not matter very much since the extent of re newal is not directly proportional to time

While the very rapid disappearance of radioactivity is disturbing in the case just discussed in other cases it may be very welcome An example is found in the application of radiophosphorus to studies of the life cycle of red and white corpuscles Such studies are based on the rapid disappearance of P^{32} from the circulation

Avian blood corpuscles contain appreciable amounts of desoxyribonucleic acid and the red corpuscles can be labeled by introducing radiophosphorus into their desoxyribonucleic acid Mammalian red corpuscles do not contain appreciable amounts of that compound which makes their study more diffi-

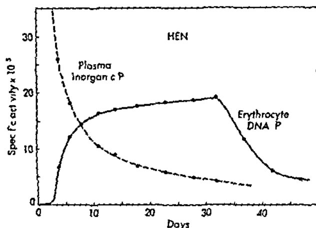


Fig 133 Specific activity of plasma and erythrocytes following injection of P in the hen

cult In Figure 133 obtained by Ottesen both the rapid disappearance of the radioactivity from the plasma and the rising activity with time of the desoxyribonucleic acid phosphorus extracted from the red cells are seen The rise occurs after two days Then the activity of the red cell desoxyribonucleic acid phosphorus remains constant for about 30 days when suddenly it decreases

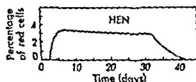


Fig 134 Percentage of red cells formed during the first day of the experiment and present in the blood stream as calculated from results shown in Figure 133

What has happened is the following after P^{32} has been injected into the hen the marrow contains high radio activity in its inorganic phosphorus During the first day or two after injection red corpsucles are found which have a high P content When these corpsules reach the end of their life cycle which is about 30 days they are hemolyzed and the phosphate of their desoxyribonucleic acid is split off by phosphatases The split phosphate is lost in the large phosphate pool of the hen Figure 134 shows the data in a slightly different fashion

We can check the results obtained above by injecting radioactive phosphate two or three times daily into the hen thus keeping the plasma activity constant We find as seen in Figure 135 that the activity of desoxyribonucleic acid obtained from the red corpuscles increases with time at first but after about 30 days becomes constant At that time all the red corpuscles present were formed during the experiment Thus the result obtained by the two different methods is almost identical

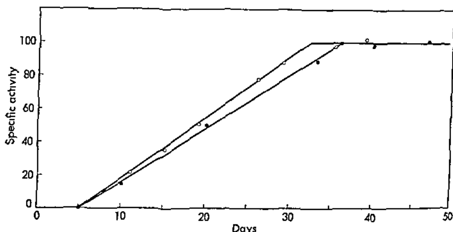


Fig 135 Life cycle of the red corpuscles of two hens Abscissa days after start of experiment ordinate specific activity of desoxyribonucleic acid phosphorus extracted from the corpuscles secured at different dates

In the determination of the life cycle of human erythrocytes Shemin and Rittenberg fed glycine containing heavy nitrogen (N^{15}) for a few days and followed the change in the N^{15} content of the isolated heme with respect to time This beautiful work led to the conclusion that the life span of adult human red corpuscles amounts to 129 days in the male and to 109 days in the female Red blood cells from a patient with polycythemia vera were shown to have a life span of 131 days and a normal pattern of red corpuscle destruction but a rate of red cell and hemoglobin production about two and a half times normal

While the determination of the life cycle of red corpuscles necessitates a type of labeling which remains in the corpuscles throughout their life the measurement of the number of red corpuscles circulating in the body can be carried out in the course of a few minutes Thus it suffices to fix the radioactive label to the corpuscles for a comparatively short time This procedure can be carried out *in vitro* We secure a sample of human blood add a few microcuries of labeled sodium phosphate of negligible weight shake

the sample for one hour at body temperature and reinject the blood into the subject. Now let us denote the number of red corpuscles injected into the circulation by *A* and the ratio of the P^{32} content of 1 gm of the injected red corpuscles to the P^{32} content of 1 gm of the red corpuscles secured from the circulation sufficiently long after the injection to ensure mixing by *B*. Then the total number of red corpuscles present in the circulation (K) is equal to *A* times *B*.

This method depends on the following facts. If to a blood sample of 10 ml kept at body temperature we add labeled sodium phosphate of negligible weight, about one third of the P^{32} atoms added are found to be present in the red corpuscles after the lapse of one hour. From this fact it follows that in the course of the hour—if one assumes the inorganic phosphorus content of the plasma to be 4 mg per cent and the weight of the plasma to be 55 per cent of the blood—about 0.07 mg. of inorganic phosphorus moves from the plasma into the corpuscles and vice versa. In the course of this interchange some of the P^{32} added to the plasma penetrates the red corpuscles and is replaced by P^{31} atoms moving in the opposite direction.

The red corpuscles contain appreciable amounts of labile organic phosphorus compounds. In the course of the glycolytic and other enzymatic processes taking place in the erythrocytes these compounds are degraded and resynthesized at a very remarkable rate. Shortly after their entry as inorganic phosphate most of the P^{32} atoms participate in the resynthesis of labile organic phosphorus compounds and are incorporated in them. The presence of a comparatively large amount of labile organic phosphorus molecules makes it possible to fix P^{32} in red corpuscles during an interval which amply suffices to carry out a determination of the circulating erythrocyte volume.

When applying this method in experiments taking more than half an hour, corrections must be applied to account for the fact that about one tenth to one twentieth of the phosphorus in the red cell is inorganic and so can exchange with the inactive plasma during the period of the experiment. Figures 136 and 137 show these facts graphically.

Nylin made a very extended application of the method described. He determined, besides the total circulating erythrocyte volume, the blood volume of organs such as lungs and legs. A blood sample of a human subject was secured and labeled with P^{32} as described above. Before injecting an aliquot of the labeled red corpuscles into the subject the legs were excluded from the circulation by means of blood pressure cuffs. The red corpuscles circulating in the legs were thus prevented from participating in the "dilution" of the injected labeled erythrocytes after these were added to the circulating blood volume.

As seen in Figure 138 10 minutes after injecting labeled corpuscles the amount of circulating erythrocyte excluding that of the legs is found from the dilution figures to be 1560 gm The removal of the cuffs leads to a marked drop in the activity of a 1 gm corpuscle sample (denoted as specific activity) Because of the participation of the red corpuscles of the legs in the second dilution process the specific activity of the corpuscles decreases from 500 to 428 indicating that the red corpuscle volume of the body which now includes that of the legs as well amounts to 1810 gm From the above figures the weight of the erythrocytes circulating in the legs is determined to be 250 gm

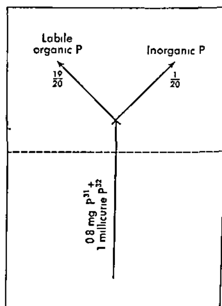
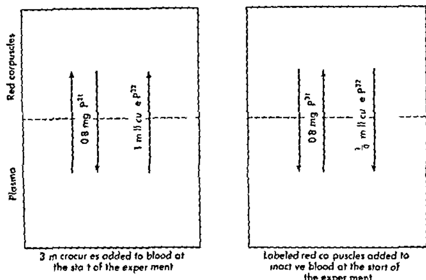


Fig 136 Distribution of inorganic phosphorus which has penetrated into the erythrocytes between labile organic phosphorus and inorganic phosphorus

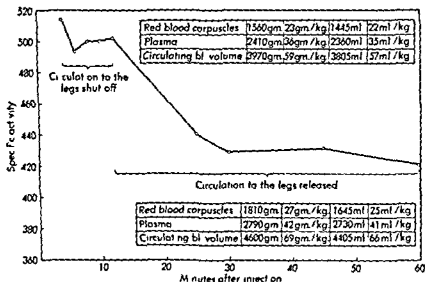
P^{32} labeled red cells have found extended application in the Donner Laboratory in recent years in the determination of the circulating blood volume in various diseases

This discussion of the life span of red cells was presented as an example of the fact that rapid disappearance of the isotopic indicator from the circulation may occasionally be helpful This is also true when studying some aspects of acetate metabolism Thus although Rittenberg and others determined the half life of acetate by feeding labeled acetate over many days until the activity in the body was constant the technique of injecting labeled acetate only at the start of the experiment yielded some additional information In this latter instance fatty acids isolated from the liver of the mouse were much less active after the lapse of one hour than after the lapse of 30 minutes This demonstrated a very rapid replacement of a minor part of the fatty acid present in the liver The labeled fatty acids first formed were rapidly replaced by fatty acid molecules newly formed in a medium containing much less radioactivity This phenomenon could only be observed by virtue of the rapid decrease in the activity level of the acetate following the single injection

One can also use this same technique to study the acceleration of metab-



F g 157 Interchange of inorganic pho phorus between plasma and red corpuscles at 37 C in the course of one hour in 100 ml of blood



Red blood corpuscles	1560gm	23gm/kg	1445ml	22ml/kg
Plasma	2410gm	36gm/kg	2360ml	35ml/kg
Circulating bl volume	3970gm	59gm/kg	3805ml	57ml/kg

Red blood corpuscles	1810gm	27gm/kg	1645ml	25ml/kg
Plasma	2790gm	42gm/kg	2730ml	41ml/kg
Circulat ng bl volume	4600gm	69gm/kg	4405ml	66ml/kg

Fig 158 Determination of the circulating blood volume of the legs (Nylin G *Am Hea t J* 34 178 1947)

olism by the dinitro compounds or the slowing down caused by such agents as urethane

CRETINISM, INCLUDING PROTEIN BOUND IODINE AND UPTAKE OF RADIOACTIVE IODINE AS AIDS TO DIAGNOSIS

Professor BARNETT (New York) It is unfortunate that as the introductory speaker for this part of the discussion I am neither a thyroid physiologist nor a radiobiologist but pediatrician and I must therefore bring the discussion to clinical problems encountered in treatment I hope that

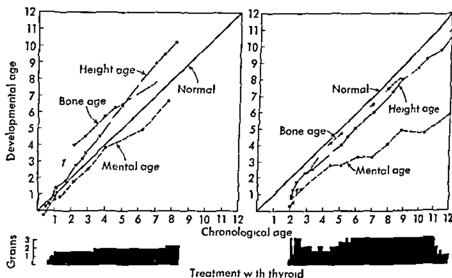


Fig 139 Growth and development of cretins treated with thyroid

Professor de Hevesy and other members of the panel will be able to tell us how the use of isotopes may help us in the problems that we encounter

In the past great efforts have been made to achieve early diagnosis of cretinism in the belief that prompt treatment of children with congenital hypothyroidism would lead to more satisfactory results in mental development However it has been common experience as shown in Figure 139 taken from a paper of Wilkins that even with very early treatment when growth in height weight and bone development proceeds at a normal rate too frequently mental age lags far behind It is even becoming evident paradoxically that the earlier treatment is started the worse is the prognosis for mental development In other words the earlier the diagnosis is made the more complete is the degree of hypothyroidism This does not however lessen our interest

in making the diagnosis early. Therefore I would like to review very briefly the present laboratory aids that we have for diagnosis particularly in the early weeks of life before symptoms become sufficiently manifest to allow easy clinical diagnosis.

The level of serum cholesterol which is of help in older children and adults is of less value in very young infants because of the frequency with which normal values are found in untreated cretins. The basal metabolic rate at least with present methods is not a practical aid in diagnosis in young infants. Until now the absence of spontaneous creatinuria in young infants has in our experience been the most useful laboratory aid to diagnosis. Bone age has likewise been a help but the frequency with which delayed bone age is found in the presence of what we have to accept as normal thyroid activity detracts from its value. Finally I should mention that the sensitivity of the hypothyroid child to thyroid extract and that the dramatic clinical response are perhaps still our best diagnostic tests.

Within the last few years the availability of two more methods has given us a greater opportunity to make the diagnosis in early infancy. These are the determination of protein bound iodine and the uptake of radioactive iodine I. Of all the chemical determinations that can be made on blood that of protein bound iodine probably comes closest to measuring the thing we want to know since the protein bound iodine is actually an approximate measure of the amount of circulating thyroxin. Values for protein bound iodine in serum have been available for some time in adults and even in children but the method requires enormous quantities of blood and this has prevented its application to small infants. In adults the method has given a normal range of about 4 to 8 μgm per 100 ml of serum. The recent development of a method for protein bound iodine which can be performed with 2 ml of serum has permitted beginning accumulation of data on young infants. I would like to say a word about this method because it has been our experience that there can be great difficulty with it.

The method which we use in our laboratory was described by Barker in 1948 and has since been modified. It consists essentially of precipitation of the plasma proteins followed by sufficient washing so that inorganic iodine is effectively removed. The second step is digestion of the protein leaving the plasma iodine in inorganic form. The inorganic iodine is distilled and determined by the iodide catalysis of the reduction of ceric ions by arsenious acid. This is markedly enhanced by the presence of a relatively high concentration of chloride which improves the sensitivity by about 35 per cent and has permitted the application to small samples of blood. Because the distillation may not be complete we have been adding a known amount of radio-

active iodine before distillation and correcting our total recoveries in relation to the recovery of radioactive iodine I think we have reason to believe this will be an important addition to our aids in diagnosing hypothyroidism in children

The uptake of radioactive iodine which has been used extensively in adults and to a lesser degree in children may also prove to be of diagnostic help in infants Our own experience with this has been extremely limited and I can just mention the two general methods of determining uptake Counting over the gland has the inherent difficulties of a geometric system for the area counted may not include the whole gland The usual procedure in adults has been to do a single count after the oral feeding of radioactive iodine at 24 or 48 hours Because there was considerable overlapping by this technique some investigators have studied the per cent increase over the base line count at 20 minute intervals for two hours after the ingestion of a single dose of radioactive iodine Their results indicate that there may be better separation of normal subjects from those with hypothyroidism by this technique than by a single count at the end of 24 or 48 hours

In these studies 20 to 50 microcuries of radioiodine were given by mouth *The results appear in Figure 140 The normal range of uptake plotted* as per cent over the base line is shown by the solid black section in which the normal range is from 125 to about 300 per cent at the end of 2 hours The values obtained in a few children with hypothyroidism show a distinct separation from the normal group and no overlapping In the very few cases with hyperthyroidism a distinct separation again appears

Dr GABRIELI (Stockholm) De Hevesy s pioneering idea was that radioactive isotopes play the same role in the metabolism of the body as the corresponding stable elements and therefore radioactive materials can be used as indicators in medical research

The use of radioactive isotopes has tempted many research workers to accept the isotope technique In the past few years much work in this field has been published but at the same time a new danger has appeared radiation damage

When using radioisotopes in research we must decide how great an activity is needed for *this* research If the activity we use is too small the determinations are troublesome If we increase the activity there is the danger of radiation damage to patients or experimental animals No comment is needed with regard to hazards to patients but even with animals care must be taken since radiation effects may alter the physiological circumstances sufficiently to modify or invalidate the research and expose the investigators to unnecessary risks It is not always easy to find the golden mean

The specific activity in the liquid or the organ that is to be examined and the sample size on which the activity is to be determined limit the possibilities of decreasing the dose We must further take into consideration the nature and energy of the radiation of the isotopes in question together with its half life and the isotope distribution in the organism All of these factors will

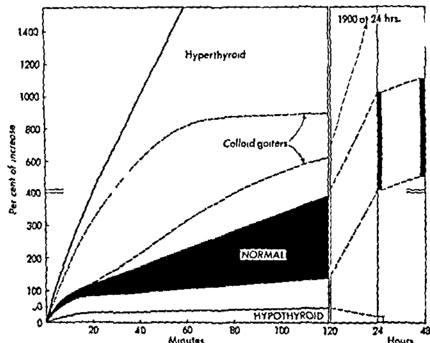


Fig 140 Radioiodine pick up curves of 1? normal children and 8 with thyroid dysfunction Ordinate is expressed as per cent increase of isotope taken up by thyroid gland with initial reading made 10 minutes after ingestion of the isotope and indicated as 0 per cent on the base line It should be noted that above 400 per cent figures are doubled

determine how great an activity is needed to perform the experiment with sufficient accuracy

From the point of view of the patient or animal receiving the isotope we must consider (1) the radiation species of the isotope its half life and what particles are emitted on disintegration and how great an energy is evolved (2) the distribution in the organism the length of time the element may remain and the physical form of the injected isotope—solid liquid, or colloidal and (3) the rapidity and routes of excretion

Professor de HEVESY (*Stockholm*) *Dr Gabrieli is right in emphasizing* how dangerous it may be to utilize radioactive agents Possible radioactive damage is a most important factor to be considered In the applications of radiosodium or P^3 labeled red cells which I described small doses were injected and the patient was exposed to less than 0 1 r e p Most studies can be carried out by using very low activities In my own experience I remember one case in which I feared that the activities applied might influence the results of the experiment It occurred when I was following the fate of P in various fractions of the organs of rats for three months This time interval corresponds to about seven periods of decay of radiophosphorus and therefore *highly active* P^{32} *had to be administered to the rat*

Professor BARNETT (New York) What is the general opinion concerning the relative susceptibility of very young infants and adults to radiation effects?

Dr LINDBERG (Stockholm) Dr Gabrieli has performed some experiments on young and old rats The young rats are much more sensitive to radiation

Professor de HEVESY (Stockholm) A great deal can be done with isotopic indicators but not everything Some people consider it sport to apply them in all sorts of research It is often a great advantage to use labeled substances but isotopic methods are in fact not too exact so that obtaining even 1 per cent accuracy is often a formidable task If a good analytical method is applicable in many types of studies it is pointless to use isotopic indicators

CHAPTER VIII

Panel on Evaluation of Nutritive Status

EVALUATION OF NUTRITIVE STATUS BY CHEMICAL AND OTHER METHODS

Professor BESSEY (Chicago) The evaluation of nutritive status may seem simple but the more one contemplates the problem the more complex it appears Before discussing the methods that are available for evaluating nutritive status their usefulness and limitations I should like to consider briefly the concept of nutritional status

Figure 141 is an illustration of the relationship between nutrition and health Here the growth rate of albino rats is taken as the measure of health and vitamin A as the example of a nutritive essential

This figure shows that as nutrition is improved with respect to vitamin A the growth rate increases up to the point where the curve levels off and there is no further benefit from further increases in vitamin A in the diet

At various points on this curve other means that one might use for measuring health are indicated The first point is labeled Normal histology If one gives 15 international units of vitamin A per kilogram daily to a rat one produces an animal which a histologist would say is perfectly normal However if this rat were examined by a physiologist he would find that twilight vision was not normal It requires around 25 units to bring about normal physiology of the rods of the retina which are concerned with vision in dim light If one accepts the contention of some investigators that storage of vitamin A is a normal and necessary process in health the intake would have to be 50 units daily to produce a healthy animal For such a criterion of health as maximum growth rate or maximum reproducibility in the females 240 and 480 units respectively of vitamin A are required

Thus nutrition is not an all-or none phenomenon There are many degrees of nutrition from a level so low that the creature does not survive up over a very wide range through which one gets added benefits to a point where one no longer obtains further benefit One must consider the range of nutrition The problem of adequate nutrition is not simple there is not a point below which nutrition is unsatisfactory and a point above which it is satisfactory Conclusions concerning the nutritive status of a subject depend on the criteria one uses for satisfactory and unsatisfactory

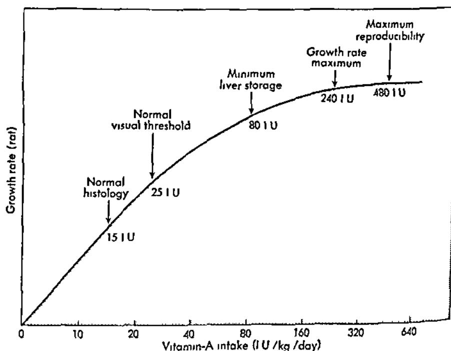


Fig 141 The nature of nutrition illustrated with data from vitamin A studies

Although I have used vitamin A as exemplifying nutrition a number of essential nutrients must be considered in evaluating nutrition as a whole Fortunately obtaining a number of these essential nutrients is not a practical problem in a good many areas so that one is not faced with the problem of evaluating all of them in order to evaluate the nutritive status of a population In most cases however it is necessary to evaluate more than one factor in order to say that the nutritive status is above a certain level

In general there are three types of methods that can be used for the evaluation of nutritive status historical and statistical clinical and chemical

methods None of these methods is completely satisfactory but each has its particular merits It is essential to define the particular problem being investigated and then use the method best suited to it Often all available methods are used indiscriminately to evaluate the nutritive status of a population without consideration of the particular method that will give the maximum information with the least effort This is unscientific

I should like to speak briefly about the historical and statistical and the clinical methods and then discuss chemical methods in more detail I think the latter in general have the broadest usefulness There are inherent limitations in the other two methods Nevertheless they are useful for certain situations

In using the historical or statistical method one determines the intake of food in a population or an individual then from food tables one determines how much of any dietary constituent is present in that intake Finally the intake of each dietary essential is compared with our knowledge of what the dietary intake of that particular nutrient should be in order to provide a given nutritive status

This technique can be applied to large population groups or to individuals For example after the First World War Chile asked the Health Organization of the League of Nations to give them advice on nutritional problems in their country They were given advice on the basis of their imports exports and production of food because the problems were of such a nature that it would have been quite inefficient to try to work with smaller groups at that time

The same method can be used for smaller areas In the United States the method has been used in mining towns where most of the food is purchased through a company store The number of people in the area is accurately known and from the books of the store the quantity and quality of food purchased in a given period can be calculated

The United States Navy has sponsored studies in which food consumed and wastage for the individual meal were weighed and calculations were made of the dietary intake of individuals This is a somewhat more precise procedure particularly if one is dealing with individuals

Clinical methods have limited usefulness since they deal with the evaluation of signs and symptoms of disease If one nourishes an animal well enough so that no signs or symptoms are evident one is apt to say that the animal is well nourished but as previously mentioned there is much evidence that there is a wide margin between keeping a creature symptom free and having nutrition provide all it can toward the health of that animal There can be acute specific signs such as those in rickets and scurvy or there can be less specific signs of deficiency disease such as the development of cheilosis

at the angles of the mouth changes in the tongue skin gums and so on I think it is fair to say that while the latter type of observation broadens the range over which we can use this method of evaluating *nutritive status there* is a loss in specificity because such things as cheilosis vascularity of the cornea or changes of the tongue are not specific signs It is quite true that these types of lesions appear more often in poorly nourished populations than in well nourished populations but a good many factors other than nutrition enter into the picture

Chemical methods are based on two general principles One is that the level of a nutritive essential in the body tissues and fluids bears a relationship to the relatively recent intake of that particular nutrient Of course dietary intake and nutritive status are not exactly the same but for most of my discussion I shall assume they are the same with the understanding that situations in which they are not the same represent special cases It is possible to have an adequate intake or a normal level in the blood of a given dietary constituent and yet not have the material properly used by the tissues In such a case one might say that in spite of adequate intake the nutritive status is not satisfactory I think such cases are rare There are of course a number of known examples in which the intake is adequate but the levels of body tissues and fluids are not satisfactory

The second general principle on which the chemical methods are based is the determination of abnormal metabolic products that result from an inadequate level of certain of the dietary essentials This principle may be subject to the same limitations as clinical methods That is abnormal metabolic products in body fluids may only appear when the dietary intake is near the point where lesions develop The analysis may be more objective however than clinical methods For if the time comes when we can detect an abnormal metabolic product let us say in riboflavin deficiency I think it probable that we can make that observation more objectively than the clinician who must decide whether cheilosis is due to riboflavin deficiency or not At the present time the only practical method of this sort is the measurement of elevated pyruvic acid levels in thiamin deficiency Since there are other causes of high pyruvic acid levels in blood this is really only an indication that *metabolism of pyruvic acid is not proceeding normally* Usually one is able to eliminate the other causes and this method has its usefulness

A few years ago the New York City Department of Health asked our laboratory to give them advice on where they could best use their efforts to improve the nutritive status of school children We made a number of studies in which we measured the level of certain nutritive constituents in the blood of groups of school children This was done at various selected areas

in the city The analyses were done by micromethods and I want to show you what we did with the data

For evaluation purposes we plotted these data on distribution curves Each of the curves in Figures 142 through 146 inclusive represents the distribution of analyses for 150 school children selected in the school designated by the letter on the curve The ordinate gives the per cent of this group of 150 that showed a level of the particular nutrient in each of the classifications shown along the abscissa To take an example from Figure 142 approximately 40 per cent of the children in school *A* had a plasma vitamin A content of from 40 to 50 μgm per 100 ml and no children in school *A* had a level less than 20 μgm per 100 ml

Four curves are shown—*A* *B* *C* and *N*—in Figure 142 *A* is from a school in an area where we know that the nutritive status is very satisfactory and that a large number of children have high vitamin A plasma levels Contrast that with school *C* which is in an area which we know for other reasons has a less satisfactory nutritive status In this group there are 35 per cent of the children in the 20 to 30 μgm per 100 ml range and 5 per cent with less than 20 μgm per 100 ml

N represents data collected by Dr O H Lowry in Newfoundland Here 50 per cent of the children had a vitamin A content less than 20 μgm per cent and practically none were above the 40 to 50 μgm per cent level

The ascorbic acid data are treated in the same way in Figure 143 In school *A* 60 per cent of the children had from 1 5 to 2 mg per cent of ascorbic acid Now these are not fasting specimens Our assumption is that not many school children changed their eating habits on the morning of the test Thus one finds children with ascorbic acid levels from 1 5 to 2 mg per cent which simply means they had citrus fruit that morning But that too is interesting information for group *C* did not get much orange juice that morning since there are very few of them in that range One finds that 25 per cent of children in group *C* have less than 0 4 mg per cent in their blood plasma and that none in group *A* were in that condition Of the *N* group 60 per cent had plasma ascorbic acid less than 0 4 mg per cent and there were practically no subjects in the upper ranges

Figure 144 shows the distribution curves for carotene Since there are many carotenoids that do not produce vitamin A the level is not strictly proportional to vitamin A precursors but really tells one what the green and yellow vegetable intake has been Again group *A* has high levels and groups *C* and *N* have low levels

We believe from our experience that determinations of hemoglobin are more useful in evaluating nutritive status than is generally realized Of course

at the angles of the mouth changes in the tongue skin gums and so on I think it is fair to say that while the latter type of observation broadens the range over which we can use this method of evaluating nutritive status there is a loss in specificity because such things as cheilosis vascularity of the cornea or changes of the tongue are not specific signs It is quite true that these types of lesions appear more often in poorly nourished populations than in well nourished populations but a good many factors other than nutrition enter into the picture

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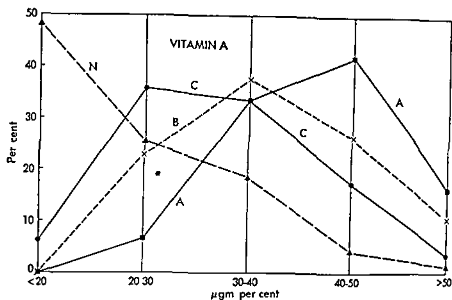


Fig 142 Plasma vitamin A content in school children of varying nutritional status

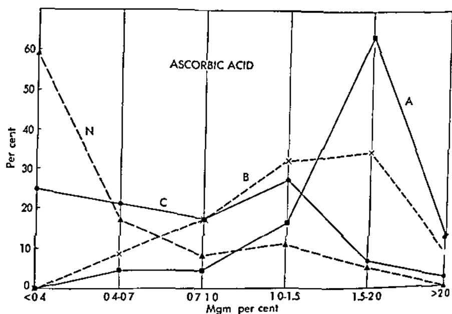


Fig 143 Plasma ascorbic acid content in school children of varying nutritional status

hemoglobin can be influenced by a number of factors only some of which are nutritional In our experience we have found a good correlation between hemoglobin levels and nutritional status provided other factors such as sex and age are properly considered It is also important that one determine the hemoglobin more carefully than is usually done in the United States for

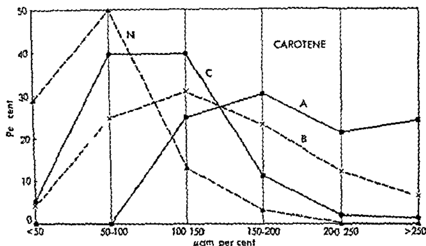


Fig 144 Plasma carotene content in school children of varying nutritional status

clinical purposes The blood specimens must be drawn at the same time of the day because there is a diurnal variation in hemoglobin levels similar to that in iron as well as a variation with muscular activity

The curves for hemoglobin distribution in Figure 145 tell the same story as is told by the previous figures These curves do not merely reflect iron intake since many nutritional factors influence hemoglobin levels nor are these levels to be considered extremely low In such studies one is not particularly interested in anemia but in whether hemoglobin levels give information about nutritive status

Figure 146 shows the alkaline phosphatase levels of the various groups In this case one is measuring an alteration in a normal physiological constituent and in nutritional deficiency the levels are higher It will be noted that group C has higher levels than group A Here again it is very important to consider sex and age in making interpretations because phosphatase varies strikingly with these factors

Figure 147 shows how great the variations in hemoglobin and phosphatase with age and sex can be

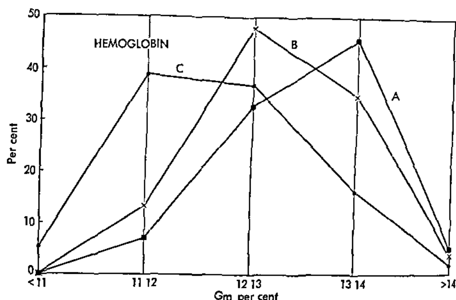


Fig 145 Hemoglobin values in school children of varying nutritional status

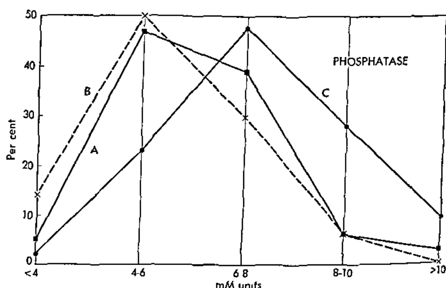


Fig 146 Serum alkaline phosphatase values in school children of varying nutritional status

As a result of such studies we were able to direct the Department of Health to those areas in which the nutritional status was lowest as a guide to their efforts to improve nutrition These methods appear to be useful for this kind

of a problem. But a further question arises from such studies: just how bad is the lowest level of nutrition we observed? This involves making an interpretation with respect to the level of nutrition as it is related to health. This is difficult because at present there is only sparse information on this point.

However, we drew up a key of interpretation based on the best information available for blood levels in terms of adequacy of nutrition. This was gathered from our own experience and from the literature. For example, we concluded that any time the vitamin A level in children of this age falls below 20 µgm per cent one is in a range in which twilight vision can be defective. This is true not only for man but for a number of experimental animals. So we selected that as the value below which we would say nutrition was poor. Then, relying on our experience as to what the blood levels are in groups which we know are well nourished, we arbitrarily graded the groups poor, fair, good and excellent. We did the same thing for carotene, ascorbic acid, riboflavin, phosphatase and hemoglobin, taking age and sex into account for the last. This key is shown in Table 30.

TABLE 30

Key to Interpretation of Blood Levels in Terms of Adequacy of Nutrition*

NUTRITIVE SUBSTANCE IN BLOOD	Indicated Level of Nutrition			
	Poor	Fair	Good	Excellent
Vitamin A †(µgm/100 ml.)	Below 20	20–29	30–49	50 and above
Carotene †(µgm/100 ml.)	Below 75	75–124	125–199	200 and above
Ascorbic Acid (mg./100 ml.)	Below 0.4	0.4–0.6	0.7–1.0	1.1 and above
Riboflavin †(µgm/100 ml.)	Below 2.5	2.5–2.9	3.0–4.9	5.0 and above
Hemoglobin (gm./100 ml.)—Females (all ages)	Below 11.0	11.0–12.9	13.0–13.9	14.0 and above
—Males (below 13)	Below 11.0	11.0–12.9	13.0–13.9	14.0 and above
—Males (13 and 14)	Below 11.5	11.5–13.4	13.5–14.4	14.5 and above
—Males (above 14)	Below 12.0	12.0–13.9	14.0–14.9	15.0 and above
Phosphatase (Nitrophenol units/100 ml.)	Above 8	Below 8 units—Satisfactory		
Serum Protein (gm./100 ml.)	Below 6.0	6.0–6.4	Above 6.4—Satisfactory	

* Bessey, O. A., and Lowry, O. H. Nutritional Assay of 1,200 New York State School Children, in *Meals for Millions* (final report of the New York State Joint Legislative Committee on Nutrition), Albany, 1947, p. 175.
† µgm./100 ml. = micrograms/100 milliliters.

15 0

Hemoglobin (grams per cent)

■ Average hemoglobin males
• Average hemoglobin females

14 5

14 0

13 5

13 0

60

Alkaline phosphatase (units)

■ Average phosphatase males
• Average phosphatase females

50

40

30

20

11 12 13 14 15 16 17 18

Age (years)

We were then able to estimate how many children of these groups could be classified as having poor nutrition how many as fair and how many as good However one really needs a great deal more information relating blood levels to dietary intake on the one hand and to health on the other before one can be as certain about this kind of interpretation as one would like But this should not delay the use of present knowledge providing that it is applied cautiously One must always recognize the limitations of present knowledge in reaching conclusions in this field

Just before the end of World War II we were asked by the City of New York if we had any means by which we could tell whether or not the nutritive status of certain groups in the city was deteriorating

For this kind of a study one needs to know whether if one collected another set of specimens from the same school after a period so short that it could be

TABLE 31

Comparison of Analyses Done on Two Specimens of Blood Taken under Similar Conditions from 95 Individuals at an Interval of One Week

	Mean 3/18/47	Mean 3/25/47	Standard Deviation
Protein (gm %)	7 34	7 38	0 16
Vitamin A (μgm %)	33 3	35 8	3 41
Carotene (μgm %)	124 5	124 8	8 39
Ascorbic Acid (mg %)	0 74	0 77	0 1?
Phosphatase (units)	1 97	1 99	0 21

assumed that nutritive status had not changed one would obtain the same analytical results and conclusions as previously In other words what is the reproducibility of our over all operation in selecting a sample of children collecting specimens and doing the analyses in the laboratory? To answer this question the study summarized in Table 31 was done The over all reproducibility is quite respectable particularly since the two sets of determinations were not made on the same children We had planned to repeat such studies in certain selected areas at regular intervals as a means of determining whether the nutritive status was changing when the war ended and the problem resolved itself

The microchemical methods are also useful for certain special problems

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Fig 147 Variation of blood hemoglobin and serum alkaline phosphatase with age and sex (Bessey O A and Lowry O H Nutritional Assay of 1 00 New York State School Children " in *Meal fo Millions* [final report of the New York State Joint Legislative Committee on Nutrition] Albany 1947 pp 183 and 187)

We had one such problem arise in connection with our survey In one high school about 15 per cent of the girls were found to have hemoglobin levels of 11 gm per 100 ml or below in an age group in which the levels should have been 13 5 gm per 100 ml Why were these hemoglobins low in these girls and what could be done about it?

We selected about 135 of the girls from this school with hemoglob n levels below 11 gm per 100 ml After thorough study by the hematologists we

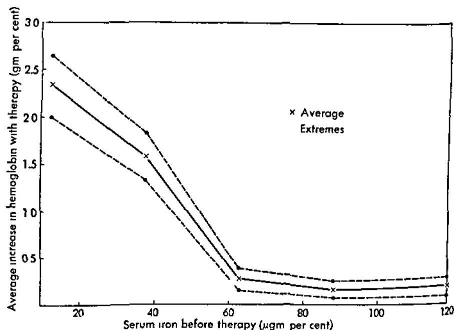


Fig 148 Relationship between serum iron concentration and response to iron therapy

examined the levels in the blood of various nutritional elements including serum iron Then the girls were put on iron therapy under the direction of a nurse We measured the hemoglobin response in this group of young women over a period of about two months Figure 148 shows the results In all subjects with serum iron levels above 60 μgm per 100 ml before the study started there was no response to iron therapy as measured by hemoglobin response In other words all the girls in this group were not iron-deficient if one accepts a therapeutic response as a measure of deficiency In subjects with serum iron levels below 60 μgm per 100 ml there was a very nice re sponse in the production of hemoglobin and in a great many their level rose from 11 to 13 5 gm per 100 ml

This was informative in several respects It told us first that about a third of the girls with low hemoglobin had serum iron levels below 60 μgm per 100 ml and for the most part they responded to iron Therefore two thirds of the girls had low hemoglobin levels because of other causes It was surprising to us to find so many young women in New York City with anemia due to iron deficiency

The second point we learned was that serum iron measurements have diagnostic value and that the critical level for serum iron at least in this age group is around 60 μgm per 100 ml As I pointed out in the Panel on Metal Metabolism when serum iron was above 60 μgm per 100 ml the level varied greatly The same individual one day might have a level of 75 μgm per 100 ml and the next day 120 μgm per 100 ml So with a serum level above 60 μgm per 100 ml one cannot tell how liberal the iron intake is However the variations in individuals from day to day when levels were below 60 μgm per 100 ml of serum were small

At the conclusion of the war the American occupation forces in Europe had a special problem in one area of Germany Rations of potatoes and beans were being distributed and some sections of the population claimed that they were not receiving their potatoes A survey team went into the area and took blood samples from several hundred subjects selected on the advice of statisticians and ascorbic acid levels were determined Since the only source of ascorbic acid this population had was potatoes if they were getting the potatoes their serum ascorbic acid would be high and if they were not it would be low Serum ascorbic acid was found to be satisfactory When the people with whom the occupation authorities had to deal were faced with this objective evidence their whole case collapsed and they admitted that they were trying to get more potatoes by false claims because everybody else was doing it also I cite this as an example of selecting methods to answer a specific problem in nutrition

I should not like to leave you with the impression that the chemical methods are the answer to all problems in the evaluation of nutritive status There are limitations One must of course first have good analytical methods But there are limitations in the range over which such methods are useful For example if the ascorbic acid intake is more than about 50 to 75 mg per day the kidney excretes ascorbic acid and maintains the blood level near 1 1 mg per cent Therefore the ascorbic acid method is useful for evaluating dietary intake only from zero to about 70 mg per day The limitation in this case is not a serious handicap since our interest nearly always lies in this range

Thiamin is an example of a lower limit If one analyzes the thiamin

content of red blood cells (and one must use red blood cells because the plasma content of thiamin is too low for analysis even with micromethods) of subjects with indubitable clinical beriberi of subjects with questionable beriberi and of subjects from the same family, with no signs of beriberi at all, one finds levels about the same in all of them As the intake of thiamin is decreased at first the content drops in the tissues including the red blood cells but at the point where abnormal metabolism is about to result in the development of lesions levels are already so low that they cannot drop much lower without death of the organism Chemical methods will not yield information in that range because chemically there is no difference between the patient with outright beriberi and the one who does not have it at the moment

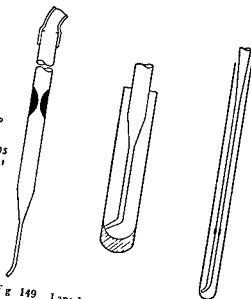
Ascorbic acid is also a lower limit example If plasma ascorbic acid drops below about 0 3 mg per cent the analysis is not very reliable and analysis of the white cells is required for information about nutritive status Plasma is not always used for analyses and one must know the physiology and biochemistry of what one is dealing with in order to select the best phase for blood analysis Heretofore we have not made full use of red cells and white cells for analytical purposes

What do we need to make our methods more useful? We need to know first more about the relationship between dietary intake and the levels in the tissues This would permit us to interpret blood analyses in terms of dietary intake with more confidence Second we need more complete information on the relation of the intake of each dietary constituent to health This is a very large project indeed because it not only involves the complications of evaluating nutritive status but it involves the very complex problem of evaluating what we mean by health

I would like to discuss briefly some of the principles used in developing the methods I have mentioned and indicate the procedure in two or three instances in order to acquaint you with their general nature

Microchemical methods are now available for vitamin A carotene thiamin riboflavin ascorbic acid plasma protein serum iron phosphatase and hemoglobin which require such small quantities of blood that adequate specimens may be collected from the finger tip thus doing away with the necessity for a venous puncture A vitamin A and carotene analysis requires but 60 cu mm of plasma ascorbic acid can be determined on 10 cu mm of serum or the white blood cells from 100 cu mm of blood thiamin and riboflavin can be measured on 20 cu mm of red blood cells etc These methods require less work than corresponding macromethods and have a precision which is as good or better

One of the first requirements for practicable and precise analysis on such



F g 149 Lang Levy pipette

Fig 150 Cuvette holder and cuvettes for microcolorimetric measurements.

a scale is a means of measuring small volumes We have used the Lang Levy pipette pictured in Figure 149 This wonderful little tool was developed in the laboratory of Dr Linderstrom Lang in Copenhagen With it it is possible to measure and transfer small volumes of specimens and reagents with remarkable precision and speed The pipettes are easy to make from small glass tubing and their use is straightforward and simple Special shapes can be made to deliver specimens to small tubes Such volume measurements are both more rapid and more precise than measurements of the usual volumes with the usual types of pipettes

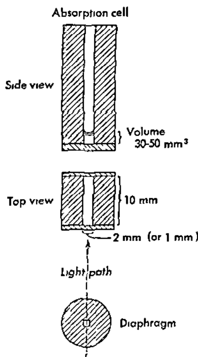


Fig 151 Diaphragm and cuvettes for microcolorimetric measurements

Colorimetry is recognized as an analytical procedure capable of a high degree of sensitivity and practicability An attachment was devised for a standard instrument the Beckman spectrophotometer which permits the measurement of color with volumes as small as 30 cu mm with essentially the same precision as previously obtained with several milliliters The attachment which fits readily into the instrument to provide a small beam of parallel light the adjustable cuvette holder and the cuvettes are pictured in Figures 150 and 151

Details of the attachment and its use as well as the details of other tools instruments and methods may be found in the reference cited at the end of this paper Colorimetric methods are used for carotene vitamin A ascorbic acid serum iron phosphatase and hemoglobin

Fluorescence is a property possessed by a number of substances For this reason it can serve as a very sensitive index of the amount of material present Riboflavin and thiamin can be measured in a very small quantity of blood by fluorescent methods with the use of a newly developed microfluorometer My colleague Oliver H Lowry has designed this instrument which is capable of measuring incredibly small quantities of fluorescent materials e g 0 1 to 0 5 millimicrograms riboflavin

A very sensitive and practicable method for serum phosphatase has been developed by the use of a new reagent p nitrophenyl phosphate The

method and the reactions involved are indicated in Figure 152 With this reagent and procedure two technicians can run several hundred determinations in a day It is clear that population surveys become feasible with such methods

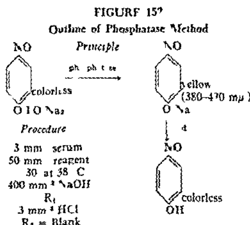
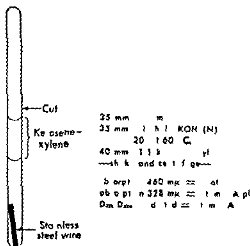
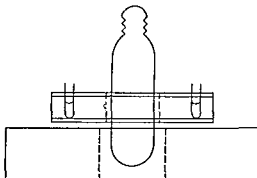


Figure 153 briefly describes the procedure for the carotene and vitamin A analysis of serum The principle is based upon the observation of Chevallier that ultraviolet light will destroy vitamin A Therefore the light absorption at 328 mμ of extracts of serum is measured before and after ultraviolet irradi



F g 153 Outl ne of carotene and v tamin A method

ation The procedures are carried out in the small tube The steel wire i for purposes of mixing as the tubes are shaken during the extraction Figur 154 shows the arrangement used for the destruction of the vitamin A b irradiation



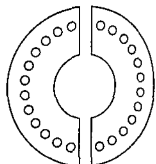
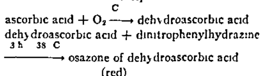


Fig 154 Arrangement for ultraviolet irradiation (Modified from Bessey Lowry Brock and Lopez)

FIGURE 155

Ascorbic Acid Determination

Principle



ascorbic acid + O_2 —C→ dehydroascorbic acid

dehydroascorbic acid + dinitrophenylhydrazine —3 h 38 C→ osazone of dehydroascorbic acid (red)

Procedure

10 cu mm serum
+40 cu mm 5% trichloroacetic acid

---

30 cu mm aliquot
+10 cu mm copper sulfate thiourea dinitro phenylhydrazine reagent

---

incubate 3 hours at 38 C

+50 cu mm 65% H_2SO_4—read at 520 mu

Figure 155 gives an outline of the procedure for ascorbic acid analysis of serum

Figure 156 gives an outline of the method for determining ascorbic acid in white blood cells

In this method a means of separating white blood cells from small quantities

FIGURE 156
Analysis of White Blood Cells Plus Platelets

100 cu mm blood
500 cu mm 1 4% oxalate

---

slow centrifugation

---

supernatant transferred and centrifuged hard

+40 cu mm 5% trichloroacetic acid to pellet of w b c

---

transfer 30 cu mm of extract for ascorbic acid determination

---

acid insoluble phosphorus determined on residue

of blood is used The same procedure has been used to isolate white blood cells for riboflavin and thiamin analyses The free flowing blood is mixed with 5 volumes of oxalate and centrifuged slowly to separate the red blood cells The supernatant which still contains the white blood cells is separated and then centrifuged hard so that a small button of the white cells is packed on the bottom of the tube These are extracted with trichloroacetic acid and the extract analyzed for ascorbic acid riboflavin or thiamin The amount of white cells extracted is determined by a microcolorimetric analysis of the precipitate for phosphate It has been shown that the acid insoluble phosphate content of white blood cells bears a constant relation to their weight This then can serve as a convenient and reliable means of determining the quantity of white cells present when the quantity is too small to weigh

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REVIEW OF RECENT DEVELOPMENTS IN FETAL NUTRITION

Dr CLEMENTS (Geneva) Developments in the field of social pediatrics and in the more fundamental field of infant metabolism in the last few years have clearly indicated that if we are to increase our knowledge of infant metabolism we must know more about fetal metabolism than we do at the present time It is my hope in this paper merely to highlight some of the information that is at present available and to indicate what I believe to be gaps in our knowledge in this particular field

Direct studies of fetal metabolism in the human are not possible and be

cause of the difference in structure of the placenta in different zoological types it is not valid to extrapolate from results obtained in domestic and experimental animals to the human For this reason we have to collect our information in an indirect way Fortunately there are a number of sources of such information which lend themselves to interpretation The material presented here is largely drawn from the literature My own experience which confirms work already done is limited to one or two nutrients

We can study concentrations of nutrients in maternal plasma in fetal plasma if available and in the plasma of the infant at different ages It is also possible to study differences of concentration of various nutrients in umbilical venous blood and umbilical arterial blood these figures give some idea of possible mechanism of transfer across the placenta It is also possible to give mothers at various periods before delivery load doses of a nutrient and then study the effect of the load dose upon plasma levels in the mother and in the newborn infant We can also study the concentration of nutrients in the various tissues of fetuses of different ages and in the newborn infant if possible collecting information on the previous health and nutritional status of the mother

A more indirect method but still one providing some information is the assay of nutrients in human milk This side of the problem has been very extensively studied by a number of groups and we have a fairly complete picture of the level of nutrients in maternal milk under varying conditions We do know that human colostrum and transitional milk have a lower concentration of some nutrients than mature milk and that the peak concentration of many nutrients in mature milk is reached at different ages of the infant Thus for ascorbic acid the peak is reached about three days after birth of the infant and from then on the concentration remains fairly constant For the B complex vitamins the plateau is reached in a period varying from 10 to 20 days after birth This kind of information suggests that the fetus has a varying capacity for storing nutrients during fetal life against the demands of the postnatal period We could deduce that the fetus has a limited capacity for storing nutrients like vitamin C but a much better capacity for storing nutrients of the B complex I think some of the workers in Scandinavia have also drawn attention to the inability of the young infant to obtain its full calcium requirements in the first few months of life even from an optimal quantity of maternal milk if we assume that its requirements are the amounts necessary to maintain the concentration of calcium in the skeleton after birth at the same level as is present at birth This suggests that the fetus stores calcium in considerable amounts if it is given the opportunity against the demands of early postnatal life

Studies on fetal metabolism suggest that the placenta plays a role in the transfer of nutrients in at least three ways by acting as a selective barrier against the transfer of nutrients by simple diffusion and by a rather complex mechanism of three stages namely trapping the nutrient from the maternal plasma storing it and then pumping the nutrient into the fetal circulation generally—and this is the interesting thing to me—against a head of pressure

Let us consider first the selective barrier mechanism By that I mean that the placenta restricts the amount of each nutrient transferred so that it prevents an oversupply to the fetus The nutrients transferred in this way are those responsible or mainly responsible for the weight of the infant namely glucose and the lipids and also the fat-soluble vitamins For these nutrients the levels in maternal plasma are higher than the levels in the fetal plasma But the levels in the fetal plasma bear a distinct relation to the levels in the maternal plasma It has been shown for instance that the blood glucose in the infant born of a diabetic mother is considerably higher than it is in the infant born of a normal mother and that that elevation is in distinct relation to the mother s blood glucose

Vitamin A behaves in an interesting way and there is still some controversy about the mechanism of its transfer to the fetus The levels of vitamin A in maternal plasma bear no relation to the levels of vitamin A in fetal plasma Large doses of vitamin A given to the mother at varying times before delivery have no effect on the level of vitamin A in the fetus Furthermore the level of vitamin A in umbilical venous blood is the same as the level in arterial venous blood suggesting that there is no transfer of vitamin A across the placenta To many of us this may be a difficult thing to accept Carotene however behaves in a different way Large doses of carotene given to a mother are reflected in the levels of carotene in the fetal plasma the latter being about one tenth of the level in the mother

Some confirmation of these facts can be obtained in an indirect way from field studies in countries like Indonesia and New Guinea In Indonesia as you know vitamin A deficiency is a serious problem in the area of infant and child health It appears principally in infants born of mothers living almost exclusively on a rice diet with practically no and in many cases absolutely no intake of foods rich in vitamin A or in carotene In New Guinea where the population is considerably less dense the consumption of vitamin A rich foods is very little different from the consumption of the same foods in Indonesia but the consumption of carotene rich green leafy vegetables is very much higher running into 6 or 8 oz per head per day Vitamin A deficiency in infants and children in New Guinea is quite unknown

The transfer of nutrients by simple diffusion is limited it seems to water

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The transfer of nutrients by simple diffusion is limited it seems to water

and to some of the electrolytes and some of the waste materials like non protein nitrogen It is possible that calcium is transferred in this way We do know that the level of calcium in fetal plasma is from 1 to 3 m higher than the level of calcium in maternal plasma The difference is due exclusively to nondiffusible proteinate or colloid calcium the levels of diffusible calcium in the fetus and the mother are identical The levels of diffusible calcium in plasma tend to bear a relationship to the total calcium There have been a number of observations made which show that a hi h correlation exists between the total fetal plasma calcium and the total maternal plasma calcium These observations suggest that the amount of calcium transferred to a fetus depends upon the head of pressure of calcium in maternal circulation even though the actual transfer of calcium across the placenta in diffusible form is by simple diffusion

The third method of transfer is to me the most interesting in that it seems that the placenta traps the nutrients stores them and then secretes them into the fetal circulation The nutrients transferred by this method are the water soluble vitamins and many of the minerals The levels in the fetal plasma of these nutrients are significantly higher than the levels in the maternal plasma the levels in the placenta are midway between the two The levels of some of these nutrients in the maternal plasma fluctuate widely being high following the consumption of foods rich in these nutrients

Iron is an example of nutrients that are probably transferred in this way In infants born of women with pernicious anemia (with high plasma iron) the plasma iron in the fetus is considerably higher than it is in infants born of normal mothers Conversely in infants born of women with microcytic hypochromic anemia (with low plasma iron) the fetal plasma iron levels are much lower than they are in infants born of normal women

It is just possible that the last two methods I have described that is transfer by simple diffusion and by the pumping or secreting method are not two methods but variations of the same method I don t think we will be able to solve that question until we know more about the forms in which the nutrients are present in normal plasma

Evidence is accumulating that nutrients are present in the blood in several forms Even the supposedly free forms may be present in one or two different forms Iodine is an example of this There is an acetone soluble form and an acetone insoluble form and one of these forms is probably attached to a protein A percentage of serum iron is bound to a protein Is the total serum iron in a form that can be transferred across the placenta or is it only the non protein fixed form that is transferrable? I don t believe we have the information at the present time to answer this question That is why

I suggest it may turn out that all the nutrients I have discussed in categories two and three are transferred by a simple diffusion of a particular form of the nutrient present in maternal plasma

All these studies indicate the importance of maintaining the level of all nutrients in the mother during pregnancy so that there will be an adequate head of pressure in maternal plasma from which the placenta can draw its supplies A great deal of work yet needs to be done on what is the adequate intake or the minimum adequate intake during pregnancy that will assure an adequate plasma level in the mother so that the fetus will obtain its optimal requirements

The other field in which a great deal of work yet requires to be done is the storage capacity of the fetus For some nutrients like vitamin A it is obvious that the fetus can store in the liver what it receives On the other hand the mechanism by which it stores the water soluble vitamins is not clear We do know that there is a wide range in niacin content of the heart of full term and apparently normal infants There seems to be some ceiling that the nutrient can reach but we don t know yet what are the factors that enable the fetus to reach that ceiling

I believe that there is a good deal of material going to waste that could be used to complete our knowledge Post mortem material of stillborns and fetuses lend themselves to study of nutrient content as well as to straight pathology That kind of information collected and correlated if possible against previous maternal health and nutrition would be very valuable

Also studies on load doses to the mother just before delivery or varying times before delivery will help us to study methods of placental transmission I do however believe that some of this work is going to be held up until we know a great deal more about the actual concentration of various forms in which nutrients are present in the plasma It would seem that for a nutrient to be transferred across the placenta it must be in a simple form possibly not even attached to protein Much more work is required on this aspect of the problem

Professor AGREN (Uppsala) There are three types of methods which may give us more information about nutrition in the future isotope methods chromatographic methods and microbiological methods I should like to discuss some possibilities of microbiological methods

These appear to be most useful for determination of vitamins and amino acids and for studies of their reactions with one another The microbiological methods are easy to learn inexpensive and I think may be useful in clinical work

The underlying idea is to use microorganisms which require for their

growth the vitamins or amino acids in which one is interested These amino acids or vitamins are excluded from the medium in which the bacteria are to be cultured so that growth does not occur When the vitamin or amino acid is added in fixed amounts to the medium the bacteria grow in proportion to these added amounts This permits determination of the amount added in the unknown by comparison with knowns

In using these methods to study infants and children one must be able to use small amounts of blood It is possible at present to determine 18 amino acids in less than 1 ml of serum The vitamins can be determined in only a few tenths of a milliliter For example only 10^{10} mg which is only a few molecules of vitamin B_{12} can be determined

Dr CLEMENTS (Geneva) The World Health Organization is interested in the whole problem of the assessment of nutritive status One of the great difficulties is to select the method that should be used in different situations It is quite obvious that the methods described by Professor Bessey apply primarily to what are known as the economically well-developed countries It is not feasible to apply them at this stage to the economically underdeveloped countries

The chemical and microbiological methods tend to narrow down the region between frank malnutrition and good health In well-developed countries they are looking for satisfactory practical ways of doing this and I think these methods are a real contribution

At the moment that is not our problem in the international field The problem is to find methods that can be applied to the less well-developed countries Robert Harris has been advocating for some time a dietary survey method as a complement to his method of analyzing the chemical composition of foodstuffs He believes the latter is important because there are foods in different parts of the world the composition of which we do not know

In 1947 the institute for which I was then responsible was given the task of carrying out a health and nutrition survey in New Guinea In one mountain village the total protein intake was 20 to 30 gm per day per person This was determined by weighing the food intakes of families throughout the whole day for a series of days Practically all of the protein was of vegetable origin Clinically the only observable abnormality was that the children from about 4 to 15 years of age seemed thin and had relatively poor muscular development but were nevertheless very vigorous and active The interesting point of the study however was that by the gradient tube method the serum protein level of the villagers was statistically higher than that of the white members of the survey party

The problem I present for discussion is this with an unsatisfactory protein intake we have adequate nutritive status as judged by an accepted method of evaluating protein nutrition

Dr BRAESTRUP (Hellerup) The interesting things we have heard tend to make one very careful in selecting standards I am sure that at least some of the standards suggested by Professor Bessey would not be applica ble to conditions in Scandinavia For instance 0 7 mg per 100 ml the border level of what you consider good as far as ascorbic acid is concerned is not reached by any number of school children from about Christmas until the strawberry season in June Dr Clements point makes one dubious about sticking to those relatively high standards

Professor RÄIHÄ (Helsinki) We have the feeling that phosphatase val ues in Finland are higher than the phosphatase values from Southern Sweden and Denmark We wonder whether this is due to lack of vitamin D or whether it is due to the constitution of the people I would like to ask Pro fessor Bessey whether the increasing phosphatase values in boys of 11 to 12 years of age can be changed with vitamin D

Professor BESSEY (Chicago) I have no information as to whether dif ferences in phosphatase level are caused by geographic factors race or en vironment I should think the thing to do would be to set up a study in Fin land in which you could select your subjects so that age and sex were taken into account Then treat a test group of these subjects with vitamin D and compare phosphatase levels with a control group Nothing answers the question about whether you need more of something as well as therapeutic response

Professor PLUM (Copenhagen) There seems to be a tendency in the United States to feel that the optimal intake of protein is higher than was felt before and yet there is considerable evidence that a lower protein intake may be satisfactory What do we know of the advantages and possible dan gers of giving high animal protein intake?

Professor BESSEY (Chicago) It seems to me that in the past we have not had a balanced view about the vegetable protein problem Our conclu sions about the biological value of vegetable proteins were drawn from a lim ited number of analyses of plant proteins that gave a distorted picture of their amino acid make up We can no longer be as certain as we seemed to have been in the past that there is something inferior about all vegetable proteins

I think there is a general tendency in places like the United States to be come interested in the greatest advantages that one can obtain from nutrition The standards are thus placed higher and higher always with eyes on the upper part of the nutritive status curve Since we have not all the information

we ought to have values that represent perhaps the optimum level based on some physiological success are selected and doubled for the sake of insurance

The so-called standards in the United States are recommended allowances In a country where it is possible to have ample food and in the absence of completely satisfactory knowledge as to what the optimum is, there is a tendency to make this allowance higher than may be necessary No one is really sure that a level of ascorbic acid in the blood of 1 mg per cent is any better than 0 5 mg per cent I do think that below that level better evidence appears that the level ought to be higher But I think that we do tend to recommend ranges that are perhaps oversafe

Now about the dangers For years there have been questions about the possible damage to kidneys which must handle too much nitrogen As far as I know until one approaches 30 and 40 per cent protein diets most of the evidence we now have does not support the view that there is danger If one gets to such levels—a pretty difficult thing to do—then I think there is difficulty I know of a case in which death resulted from too much protein—around 50 per cent of the intake for about a week Some of it was given parenterally and I am certain that death resulted from this The Eskimo can tolerate a level of proteins very much higher than those of us who live farther south without this intoxication

CHAIRMAN There is a paper from Denmark about Seventh Day Adventists who eat no animal protein In a study made by a Danish physician it was demonstrated that those people had a much lower resistance to tuberculosis

DR BRAESTRUP (Hellerup) They were also at a border line level in daily caloric intake in the 15 to 25 age group

Dr CLEMENTS (Geneva) Perhaps two thirds of the world s population are in situations such that for a long time to come they and their children will be unable to obtain intakes of animal proteins anywhere near the amount recommended for economically developed peoples I refer to the populations of Asia Africa and South America There is very little mammalian milk or animal protein in those areas and very little opportunity of producing it We are trying to collect information about diets in such areas which do not contain animal protein and which seem to have been satisfactory particularly before they were distorted under western influence An example of this distortion followed the introduction of the idea that milk is good Little milk was available but cereal gruels were substituted for important parts of the native diet principally because gruel looked like milk

Most investigations of vegetable protein have centered on grains which

contain obviously incomplete proteins Recent evidence suggests that proteins from leafy vegetables may be complete First children in Central America raised on a diet of maize beans and leafy vegetables do quite well by ordinary standards and much better than infants raised on maize alone Second civilians in a prison camp during the war under the direction of intelligent Malayan medical officers consumed 8 to 16 oz of green leafy vegetables daily and no animal protein Their general nutritional status was better when they left the camp than when they went in

As for amounts of protein the work of Stare and Hegsted showed adequate protein nutrition could be maintained on 35 gm daily in adult males and our New Guinea work suggests this is true even for pregnant females However this intake though producing excellent infants in New Guinea did not seem adequate for adolescents The adult human does not seem to need as much as our Western standards recommend but perhaps the growing child does

Dr JOSEPHSON (Stockholm) Dr Testerman was a prison physician in Helsinki shortly after the war and sent serum from about 25 healthy prisoners and serum from 25 well nourished Finns to Professor Dahlberg and myself The food situation in Finland at that time was rather poor and these criminals received a relatively poor diet containing a barely sufficient number of calories

The serums were examined for all the usual blood constituents which are determined in clinical laboratories It was remarkable how little difference there was between the serums of the prisoners and the controls The prisoners had higher levels of serum phosphatase very slightly *higher* serum proteins and lower serum cholesterol levels than the controls There were no other important differences

Professor STEARNS (Iowa City) We were asked by the State of Arizona to make studies on the utilization of iron from beans because the large number of mothers of Mexican origin feed their babies skinned and mashed beans at about 3 months of age The pediatricians were a little horrified although they had to admit that the Mexican babies did not have anemia while anemia was very common in babies of the white population in Arizona We found too that the skinned and mashed beans were an excellent source of iron Beans were a food habit that had apparently come down through the ages A young physician from Brazil brought to Iowa City several varieties of Brazilian black beans and we found that infants and young children used the protein and iron very well from these beans too

Dr Joseph Johnston of Detroit has been studying the protein requirements of adolescents for a number of years He has been studying a group of institutionalized boys and has found that the caloric intake differs very widely

from boy to boy and seems to depend upon his rate of growth at the moment However if the protein intake is kept at 15 per cent of whatever the total calories are the boys remain in excellent physical condition and maintain a *good proportion of muscle while growing* If the protein is dropped much below that the boys are like children who are up to normal height and weight but are not clinically good

Professor PLUM (Copenhagen) In Denmark we teach the students and nurses to tell mothers that infants should receive some meat fish and eggs every day from about six months on Can anybody tell me why?

Professor BESSEY (Chicago) We know with reasonable certainty that if we feed meat fish and eggs we get a reasonably well balanced mixture of amino acids More knowledge is needed to determine whether cereals or any other source can be substituted to supply essential amino acids In your country where supplies of fish and milk are plentiful feeding them is naturally the easiest thing to do If vegetable proteins are substituted without full understanding a distorted situation may result as Dr Clements has pointed out I think this same point applies the world over We have a tendency as Western people to try to have the whole world use milk whereas as Dr Clements has said a large part of the world cannot have milk So a great deal more knowledge of what can be used instead of milk is required and the knowledge we have should be used more completely than has been the case

Dr CLEMENTS (Geneva) I think the pattern of existing dietary habits must be followed to a large extent in solving nutritional problems In Denmark and other Western countries meat fish and eggs form the basis of the adults diet They like it and know it is good You will certainly disturb the mothers of such a population if you try to teach them to wean their babies to beans and green leafy vegetables unless you can alter the diet and economy of the entire country

CHAIRMAN You mean that in every country the natives have chosen by intuition and heredity those of the foods which are available through which they will fare well and remain healthy?

Dr CLEMENTS (Geneva) The dietary pattern of an indigenous population is the pattern of the people who survive

Professor BESSEY (Chicago) I do not believe that what a people has chosen and developed in the way of food customs is necessarily a good index as to what is nutritionally good for them But if it is not good and you try to change their dietary habits you have to try to change them along lines that do not radically change these customs For example the development of a nutritionally better variety of corn for use in a corn-eating area is a far more *feasible plan than a change from corn to wheat* We know from experience

that you cannot easily change the customs of large groups of people from eating corn to eating wheat In these areas that Dr Clements has been discussing efforts to improve food are best realized by studying the nutritive values of the foods they already produce and encouraging production of better foods of these types without making drastic changes in the pattern of the economy or food habits

Professor YLPPÖ (Helsinki) I remember two cases in which mothers had very serious malnutrition during the last trimester of gestation and in spite of this the infants were quite well One mother had carcinoma of the stomach and the other carcinoma of the breast Both were invalids and died shortly after delivery by Caesarean section

During the war we saw a lot of young refugee mothers and their infants Many mothers were so sick and weak that they could not walk and many had severe scurvy with large hemorrhages in the skin The whole breast of one mother was quite yellow and dark from subcutaneous bleeding Yet the babies were all breast fed and looked quite well The older children however were as weak as their mothers and many of them could not walk Thus in the last days of gestation the fetus is provided with essential nutrients regardless of the health of the mother

During the First World War we made some experiments in which we divided young pigs into three groups One was fed only protein another was fed only carbohydrates and the third group only fat as far as it was possible to give only fat To all three groups we administered the same dose of tubercle bacilli intravenously on the third day of life As the pigs died or were killed we found at post mortem that the pigs fed carbohydrates only had the most advanced tuberculosis and those pigs fed protein only lived longest with the mildest symptoms

CHAIRMAN Your first point demonstrates excellently what obstetricians say the fetus is a parasite upon its mother

Professor PLUM (Copenhagen) Practically all our national income in Denmark comes from selling our meat and butter The most profitable way for us is *not* to give meat fish and butter to our own population but to export it so that we may import other goods Now in Denmark the people generally used to give their infants for the whole first year cereals and milk The medical profession with a good deal of feeling has forced people to give their children a more varied diet including meat and fish It would be very useful to know whether this latter diet or a carefully composed vegetable diet is better I quite agree however that this is difficult to do and the safest way is to recommend a diet with meat and fish

Professor STEARNS (Iowa City) In our country the northern states are

dairy producers and there is very little milk in the southern states where nutrition is poorest So the federal government is trying the experiment of bringing in dried skim milk of which we have an excess and of encouraging the northern states to use something else for feeding young livestock The best way to get this milk accepted as a new article of diet by southerners is to use it in school lunches If the children once accept it and are taught that it is highly desirable they will insist that the families use it Its use has made a very real improvement in the nutrition of southern children and it is going to be used on a much larger scale

In the United States we use either egg yolk or meat if families prefer it as sources of iron for young infants What do you use for infants who are over three months and have largely used up their own stores of iron and are not getting quite enough from milk?

Professor LINDERSTRÖM LANG (Copenhagen) It appears to me that besides the question concerning essential amino acids and the difference between vegetable and animal proteins there is also the question of what foods are given with the proteins We have talked about the Eskimo who can handle much protein Is this connected with the fact that they eat much more fat at the same time? When children are taught a certain diet perhaps their physiological system adapts to a certain mixture of foodstuffs so that they can live more easily on that

Dr CLEMENTS (Geneva) I should like to take up the point raised by Professor Plum Most of the balance studies suggest that somewhere between 3 and 4 gm of protein per kilogram of body weight gives the best growth I think it becomes a question for each country to determine what is the most satisfactory way of providing that amount of protein during the latter part of the first year and the toddler age period I attended a well baby clinic in probably the poorest area in Amsterdam The physician in charge was giving beans to the infants as a supplementary source of proteins from about four months on This physician met the economic situation by using the cheapest form of protein together with cow s milk and when these children were weaned it seemed to me they were very close to a balanced diet

Professor AGREN (Uppsala) Professor Bessey referred to a case where too much protein was given and death resulted Can you tell us more?

Professor BESSEY (Chicago) There are in the literature reports of deaths and severe illness occurring from the oral consumption of too much protein If more than 30 to 40 per cent of the caloric intake is protein this is a possibility The particular case I mentioned occurred because someone did not know this The patient had had a high protein diet and then was given amino acids parenterally

Professor AGREN (Uppsala) Professor Bessey mentioned the possibility of developing new varieties of corn more valuable from the nutritional point of view I think I have seen a report of an investigation from the United States in which a complete amino acid analysis of several varieties of corn had been made On the whole the figures for the different species of corn agreed fairly well

When you discuss vegetable versus animal proteins where does the animal factor enter? I have seen a paper from India where animal proteins are forbidden because of religious reasons and there is much nutritional anemia because of this Since there are many populations which do not consume any meat there ought to be quite a high percentage of such types of anemia in these people

Professor BESSEY (Chicago) A species of corn has been produced in which the niacin content is higher than usual Although I recognize that there must be limitations to experimental genetics I would say that if a change in amino acids was not found in 12 varieties of corn further search should be made

Dr CLEMENTS (Geneva) In Amsterdam Professor Jansen told me that vegetable proteins contain the animal protein factor vitamin B

Dr BRAESTRUP (Hellerup) If the selection of a diet is left to the mothers it has been my experience in Denmark—and in some parts of Sweden—that they will select the cheapest and most easily prepared diet they can So the main problem is not just teaching them to change their dietary habits if you have to do so for nutritional or economic reasons but to make it *easier* for them to use the food which is considered the best Of course we still have the formidable task of proving what actually is the best In considering the economic and educational aspects I feel that it is indispensable for us to agree on scientific requirements which are as *low* as we consider safe before we approach legislative bodies

CHAIRMAN I supposed we were referring chiefly to retarded areas when talking about the choice of foods and the economic side of the question

Professor RAIHA (Helsinki) If we secure optimal growth with a well balanced diet we shall have a population of large-sized people They must live under conditions where they can only obtain 1800 calories as Dr Clements showed and yet such a population might require 2500 calories What is optimal growth then?

Professor LEVINE (New York) The difference between vegetable and animal proteins depends upon the amino acid content Furthermore as I think Professor Linderstrom Lang intimated not only must the amino acids be present in optimal amounts but their rate of utilization must be identical

Paul Cannon among others has pointed out that even if all of the essential amino acids are given and they are not utilized simultaneously a law of the minimum operates and none of the essential amino acids will be utilized in the absence of utilization of a single one

Again as Professor Linderstrom Lang said proteins are not metabolized alone There have been demonstrated innumerable interrelationships between proteins amino acids vitamins carbohydrates and fats and these are relevant to the problem of nutrition

Do we know that the optimal protein intake in different countries is the same? *The constitution of the individual in different countries may play a* role and the growth of children in different countries indicates that it does A substance like cortisone increases protein anabolism and thyroid increases protein catabolism Different populations may have developed different hormonal constitutions throughout the ages which make a particular type of diet more efficient for one group than for another A proven nutritional optimum for one country is not necessarily optimal for another

CHAIRMAN The choice of food in underdeveloped countries depends on the choice of food of those who survived Perhaps the survivors were those to whom individual differences gave a physiology which could digest absorb and utilize that kind of food

Professor BESSEY (Chicago) It seems to me that there are so many factors involved in nutritional requirements and in the conditions under which those requirements must be supplied that nothing more than base line figures can be given Each problem must be considered from the scientific economic sociological and cultural points of view One s desire to be on the top part of the nutrition curve may have to be balanced against economic factors There has been a tendency in the field of nutrition to have rules like a quart of milk a day We ought to have instead perhaps the philosophy an engineer has *Each problem is a different one* and must be considered in the light of all available knowledge

Professor BARNETT (New York) Ultimately we are feeding infants and children A problem we have in the United States partly independent of what the child *should* eat is what he *does* eat *Many of our children* who are offered what we consider to be an optimal diet are in some instances undernourished because of psychological factors Dr Marriott once came back from a conference on nutritional treatment of marasmic infants and saw a baby who was well but not eating *He said that if that baby could have* been at that conference he would probably begin to eat This is a serious nutritional problem in certain parts of the United States

CHAIRMAN I suppose that is true not only in the United States

CHAPTER IX

Panel on Metabolism in Premature Infants

METABOLISM IN PREMATURE INFANTS

Professor LEVINE (New York) In any discussion of infant metabolism the problem of prematurity deserves a high priority Approximately five of every one hundred live born babies are premature births Yet this 5 per cent accounts for practically 50 per cent of all neonatal deaths This high premature death rate may be lowered by good obstetrics skillful nursing avoidance of infections maintenance of body temperature preparedness for emergencies and proper feeding the subject of this discussion

Appetite is a good gauge of the adequacy of intake in full term infants who are strong enough to suckle the breast directly or to take the bottle They can usually be relied upon to ingest sufficient amounts of food to satisfy their metabolic needs Insufficient food leads to irritability sleeplessness and crying excessive food to refusal and if feeding is persisted in to vomiting

These criteria are of little value in premature infants They are often too weak to suck spontaneously and they frequently become exhausted before completion of feedings When fed by gavage they must accept whatever is offered Criteria other than appetite and subjective behavior are therefore needed to determine the kind and amounts of food which best meet their nutritional requirements This information is obtainable from metabolic and balance studies

In this introductory talk I propose to present a description of the apparatus and technique for making metabolic measurements in premature infants Some of the results obtained by use of these methods will also be described

Paul Cannon among others has pointed out that even if all of the essential amino acids are given and they are not utilized simultaneously, a law of the minimum operates and none of the essential amino acids will be utilized in the absence of utilization of a single one

Again as Professor Linderstrom Lang said proteins are not metabolized alone There have been demonstrated innumerable interrelationships between proteins amino acids vitamins carbohydrates and fats and these are relevant to the problem of nutrition

Do we know that the optimal protein intake in different countries is the same? *The constitution of the individual in different countries may play a* role and the growth of children in different countries indicates that it does A substance like cortisone increases protein anabolism and thyroid increases protein catabolism Different populations may have developed different hormonal constitutions throughout the ages which make a particular type of diet more efficient for one group than for another A proven nutritional optimum for one country is not necessarily optimal for another

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These data are presented in preparation for the consideration of the specific topics to be taken up in the subsequent panels

Figure 157 shows the methods used for obtaining an accurate estimate of intake The total *feedings for each 24 hours are weighed on an accurate* balance and fed to the infant at 3 hour intervals by bottle or gavage The

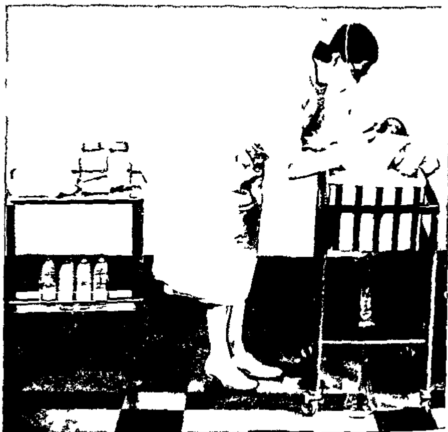


Fig 157 Methods for estimating intake in premature infants

amounts refused regurgitated and vomited at each feeding are collected in tightly sealed jars pooled weighed back and deducted from the initial weight of *the feedings The difference records the weight of food* actually ingested by the infant

Accurate timing of voidings is facilitated by a device constructed by Dr Hoag and shown in Figure 158 The specimen tube enters a crucible the base of which is perforated by a number of small holes When urine is passed it enters the crucible and weighs it down This in turn pulls on a

thread which closes an electric contact and causes a bell to ring As the urine leaves the crucible through the perforations the thread rises the contact is broken and the bell stops ringing This device saves nursing time and checks the timing and accuracy of collections Even small losses are readily noted at the time of voiding

The apparatus thus far described ensures accurate measurement of the intake and of excretion by way of the feces and urine These fractions are

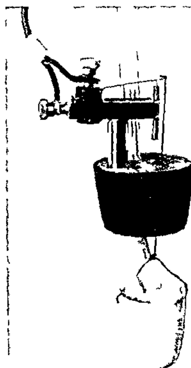


Fig 158 Apparatus for timing of voidings

chemically analyzed for the constituents under study water nitrogen fat minerals vitamins and calories if desired

Figure 159 shows the balance used for measuring the insensible perspiration or the water vaporization from the skin and lungs The infant rests on a metabolism frame and is weighed at intervals throughout the period of observation The initial weight of the infant plus the weight of the food which he receives during the study is deducted from the final weight of the infant plus the weight of feces and urine passed during the observation The differ-

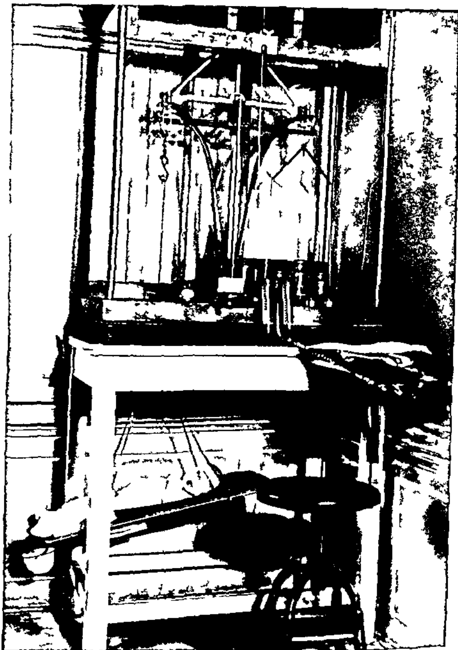
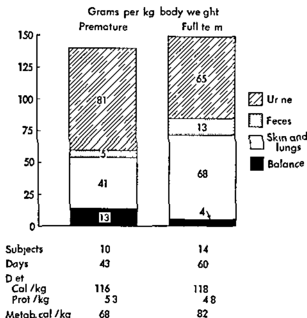


Fig 159 Apparatus for measuring insensible water loss

ence is a measure of the weight of water and CO eliminated by way of the skin and lungs

In the respiration chamber or indirect calorimeter for measuring the energy exchange of premature infants the infant resides in the chamber and samples of incoming and outgoing air are periodically analyzed for oxygen and carbon dioxide The oxygen consumption per unit of time the respira tory quotient and the urinary nitrogen afford a measure of the energy expendi



F g 160 Water exchange of premature and full term infants on adequate diets of cow s milk.

ture and organic metabolism Finally careful observation of the infant s activity from minute to minute throughout the day and night permits fractiona tion of the energy expended during sleep restlessness and crying The de velopment of these methods provides a satisfactory means of studying the water energy protein fat carbohydrate mineral and vitamin requirements of premature infants

Figure 160 portrays the results of water balance studies

Ten premature infants and 14 full term infants receiving adequate diets of cow s milk mixtures at average levels of approximately 150 ml of fluid per kilogram of body weight excreted variable amounts of water by way of the

urine feces and skin and lungs but in every instance an ample surplus was retained in the body for growth It is worthy of note that on similar fluid intakes the average water balance was of lower magnitude in the full term infants that the water loss in these subjects by way of the urine was less and that the insensible perspiration was higher than in the premature infants The lower output in premature infants by way of the skin and lungs is con

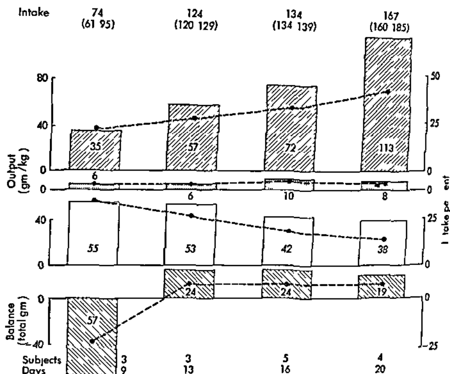


Fig 161 Average water balance of premature infants at four levels of water intake (Gordon H H "Prematurity in Levine S Z (ed) *Advances Pediat* 2 329 1947 Year Book Publishers Inc)

sistent with their low energy metabolism their diminished sweating mechanism and their relatively high rate of heat loss by radiation and convection The higher positive water balance in these subjects is consistent with their more rapid rate of body growth

Even though these levels of fluid intake yielded positive water balances the question may be raised as to whether they are necessarily optimal for premature infants An answer to this question may be obtained by placing groups of infants on different fluid intakes and determining the magnitude of their water balances

It is to be noted in Figure 161 that with fluid intakes above 120 ml per

kilogram similar retentions resulted irrespective of the level of intake The extra incoming water at the higher levels of intake was merely excreted as dilute urine the water loss by way of the feces and skin and lungs remaining relatively constant Below fluid intakes of 100 ml per kilogram of body weight the infants were all in negative water balance One infant on this curtailed fluid intake showed marked signs of dehydration and developed de hydration fever

Figure 162 correlates water retention and total body weight gain and con firms by another approach the validity of the figures proposed for fluid intake

FIGURE 162

Relation of Wat r Retention to Body Weight Change*

Subject	Days Ob	Intake per Kilogram: Calorie	Intake per Kilogram: Protein gm	Intake per Kilogram: Water gm	Water Retention gm	Body Weight Change gm	Water Wt p r cent
				Human Milk			
R L	3	141	2 1	187	13	[illegible]0	65
N O	6	118	2 [illegible]	149	20	0	67
E C	3	126	1 9	158	15	22	68
E M	3	102	4 5	135	19	29	65
41 E 4	15	122	2 9	156	17	25	66
				Cow s Milk			
N O	3	108	2 5	166	12	19	63
E M	3	120	4 8	136	23	33	70
L O	12	119	4 [illegible]	150	24	45	54
E C	4	124	6 0	130	41	65	63
H L	12	133	6 1	126	24	42	56
R L	2	134	6 3	160	43	61	70
41 E 6	36	123	5 0	145	28	44	63

* Gordon H H and Levine S Z *J Pediat* 25 465 1944

It will be seen that on these fluid intakes of approximately 150 ml per kilogram of body weight whether in the form of human milk or cow s milk mixtures the average retentions of water comprised roughly 65 per cent of the total body weight gain the precise proportions which exist in protoplasmic tissue as chemically determined

It may therefore be concluded that fluid intakes between 130 and 180 ml per kilogram of body weight are adequate to cover the water requirements of thriving premature infants and to provide a surplus for body retention con sistent with satisfactory qualitative and quantitative growth Since minimal amounts are preferred to avoid upsetting an already lowered tolerance of the gastrointestinal tract it is our routine practice to give 150 ml of fluid per

kilogram (2½ oz per pound) to all premature infants after the first week of age except in those under 1500 gm (3⅓ lb) The latter are given 130 ml per kilogram (2 oz per pound)

The next series of figures summarizes the results of studies of the energy exchange of premature infants The energy requirements comprise (a) the basal metabolism (b) the specific dynamic action of foods (c) the activity quota (d) the caloric loss in feces and (e) the growth quota

FIGURE 163

Metabolism of Premature Infants in Cal /Kg /24 Hrs
Arranged According to Age Groups*

Age Days	Number of Infants	Number of Observations	Mean Cal /Kg /24 Hrs	Probable Error of Means
1–7	11	17	52 8	1 237
8–14	11	12	58 1	1 004
15–21	9	12	62 0	1 698
22 28	8	10	62 1	1 868
29–70	5	13	59 2	1 211
TOTAL	22	64	57 9†	0 938

Gordon H H and Levine S Z *Am J Dis Child* 52 823 1936
† Coefficient of variability 11 per cent

Since premature infants will only remain quiet during sleep and following a small meal measurement of their basal metabolism really represents their sleeping metabolism plus the specific dynamic action of food One notes from Figure 163 that their average basal rate after the first week of postnatal life 58 calories per kilogram per 24 hours is the same as that for full term infants In the first seven days of life their basal rate is significantly lower averaging 53 calories per kilogram per 24 hours

Figure 164 shows their total daily catabolism as observed in 10 infants for a total duration of 124 days This quota includes not only their sleeping metabolism and the specific dynamic action of ingested food but also the activity quota The average figure obtained in these subjects of 69 calories per kilogram per 24 hours is roughly 20 per cent above their basal rate in contrast to the average figure of 82 calories per kilogram or 40 per cent above the basal rate for full term infants This difference is consistent with the markedly reduced activity of premature infants

Figure 165 shows that the caloric loss in the feces of premature infants chiefly in the form of fat averages approximately 20 calories per kilogram per 24 hours in contrast to less than 10 calories per kilogram for full term infants

FIC URE 164

Total Catabolism of Premature Infants

Subject	No of Days	Ave Weight kg	Cal Kg /24 Hrs
C	9	1 8	61
B	24	2 1	70
I	20	2 1	63
O	12	2 1	63
E	9	2	[illegible]3
M	11	2 3	2
C	11	2 4	74
I	9	2 6	65
O	9	2 6	[illegible]0
L	10	2 7	[illegible]
TOTAL AVE	124	[illegible] 3	69
	(19 per cent above basal)		
FULL-TERM			82
	(40 per cent above basal)		

FIGURE 165

Feces of Premature Infants

Cal /Kg /24 Hrs

Subject	Average		Range
C	32		20-4[illegible]
B	[illegible]5		15 9
L	14		8-18
O	16		10-[illegible]4
F	19		16-[illegible]1
M	27		18-31
C	9		9 11
P	12		9 15
O	10		7 13
L	22		22 24
		Per Cent of Basal	Per Cent of Dietary Cal
TOTAL AVE	19	33	15
FULL TERM	5-10	8 16	5-10

A more detailed discussion of fat absorption in premature infants will be taken up later

The total daily caloric need for maintenance therefore averages 90 calories per kilogram for premature infants—60 for the basal quota 10 for activity and 20 for loss in feces

In general then a caloric intake of 90 calories per kilogram will cover the maintenance requirement of most premature infants A few may require less

or more depending chiefly on the degree of activity and on the ma_nitudε of fecal loss

The caloric needs for growth remain an approximation If one assumes that the daily weight gain of 12 gm per kilogram in utero in the seventh month of pregnancy approximates that which should be gained by the pre

FIGURE 166

Calories Stored per Gram of Weight Gain

	No of Infants	No of Days	Ave	Range
Rubner and Langstein	2	20	2 9	2 ? to 3 5
Levine et al	3	6	2 7	1 7 to 3 6
Present Study	10	124	2 3	1 0 to 3 6

mature infant in early postnatal life then 30 calories per kilogram per day would be required for a daily gain of 12 gm of body weight as noted in Figure 166 It may be seen that in three separate caloric balance studies in infants the calories stored per gram of weight gain ranged between 2 3 and 2 9

FIGURE 167

Approximate Caloric Requirements of Premature Infants*

Per Kg /24 Hrs

	Mean	Range
Basal	60	50–75(a)
S D A } Activity }	10	
Total Catabolism	70	60–75(b)
Feces	20	10–30
Maintenance	90	75–100
Weight Gain	30	25–35(c)
TOTAL	120	100–135

* Gordon H H Prematurity in Levine S Z (ed) *Advances Pediat* 2 323 1947 Year Book Publishers Inc

(a) 22 infants

(b) 11 additional infants

(c) Rate of weight gain in utero—grams × 2 5 calories

The total caloric requirements of premature infants as based on these studies therefore averages 60 per kilogram for the basal quota 10 for the specific dynamic action of food and activity 20 for fecal loss and 30 for growth making a total of 120 calories per kilogram (55 calories per pound) per day Figure 167 illustrates this This caloric intake will suffice for most premature infants Only rarely will more be required for an occasional infant

with excessive activity or in one receiving a high fat intake accompanied by a fat intolerance of the alimentary tract

Figure 168 shows that premature infants have no defect in their absorption of protein from the gastrointestinal tract With protein intakes ranging be

FIGURE 168
Absorption of Nitrogen by Premature Infants* †

Type of Milk	No of Obs	No of Days	Ave Protein Intake gm kg 24 hrs	Coefficient of Digestibility‡ per cent
Human	11	69	2 3	79 (65 87)
Cow s	5	25	2 8	88 (84–92)
Cow s	21	118	4 7	91 (87–95)
Cow s	1	4	9 1	93

Gordon H H and Levine S Z *J Pediat* 25 470 1944
† Age 3–60 days weight 1 4–2 8 kg
‡ $\frac{\text{Dietary} - \text{Fecal Nitrogen}}{\text{Dietary Nitrogen}} \times 100$

tween 2 3 and 9 1 gm per kilogram per day whether in the form of human milk or cow s milk mixtures their coefficient of digestibility ranged between 79 and 93 per cent As a matter of fact in the one infant who received more than 9 gm per kilogram the coefficient of digestibility was highest Figure 169 shows that their body retention of nitrogen similarly rose with increasing

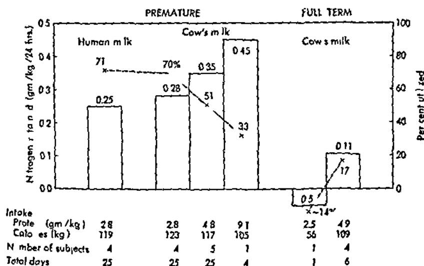


Fig 169 Nitrogen retention by premature and full term infants effect of changing intake (Gordon H H and Levine S Z *J Pediat* 25 4 0 1944)

protein intakes from 25 gm per kilogram per day retained on the low intake of 2 8 gm to 45 gm of nitrogen on the highest intake of 9 1 gm of protein

Therefore premature infants have no defect in protein utilization In view of their heightened needs for growth it is our routine practice to provide from 4 to 6 gm of dietary protein per kilogram (2 to 3 oz per pound) in contrast to the customary intake of less than 4 gm per kilogram in full term infants

FIGURE 170

Average Respiratory Quotients*

Age of Infants	No of Infants	No of Obs	Resp Quotients Average	Range
Premature				
Less than 9 days	10	18	0 88	0 77–0 95
More than 9 days	16	38	0 91	0 87–0 95
Full Term				
1–12 mos	15	19	0 88	0 77–0 94

* Gordon H H and Levine S Z *J Pediat* 25 471 1944

The ability of premature infants to utilize carbohydrates even in the first postnatal week is shown by their normal or reduced blood sugars by the absence of reducing substance in the urine and by the high levels of the respiratory quotients as seen in Figure 170 It is our practice to provide from 15 to 20 gm of carbohydrate per kilogram in the daily intake (from 7 to 9 gm per pound)

In contrast to the effective utilization of protein and carbohydrate by premature infants is their defective absorption of fat from the gastrointestinal tract Figure 171 shows that on customary fat intakes of 4 5 gm per

FIGURE 171

Fecal Excretion of Fat by Premature Infants

No of Obs	No of Infants	Dietary Fat Type	gm /kg	Fecal Fat gm /kg	cal /kg
5	4	Cow s	4 8	2 0	19
14	10	Human	6 2	1 9	18
22	7	Olive Oil	4 5	1 7	16

kilogram or more whether in the form of cow s milk fat human milk fat or olive oil the fecal excretion of fat by premature infants averages around 2 gm or 20 calories per kilogram, in contrast to less than 1 gm or 10 calories per kilogram of fecal fat in full term infants as shown in Figure 172 In none of the 10 full term infants did the fecal loss of fat exceed 1 gm or

FIGURE 172
Fecal Excretion of Fat by Young Full Term Infants

Age Months	Dietary Fat gm /kg	Fecal Fat gm kg	Fecal Fat cal /kg
3/4	5 8	1 0	9
1	5 5	0 8	7
2	5 7	1 0	9
2	6 0	0 9	8
2	6 4	0 6	6
2 1/4	5 8	1 0	9
2	4 3(a)	0 6	6
1	4 2(b)	0 2	2
1 1/2	4 4(b)	0 7	7
1	4 3(b)	0 4	3
Average		0 7	7

(a) Human milk.
(b) Olive oil

FIGURE 173
Effect of Varying Amount of Dietary Fat on Excretion of Fat

Days	Dietary Fat gm /kg	Fecal Fat gm /kg	Per Cent Dietary Calories	Weight Gain gm /kg
Infant P A			(a)	
10	1 8	1 0	9	14
8	1 9	0 8	7	15
7	4 7	1 9	16	7
9	2 1	0 7	5	16
Infant II A			(b)	
11	2 2	1 2	10	13
8	2 1	1 1	9	15
7	4 1	3 3	27	5
9	2 1	0 7	5	4

Gordon H H and Levine S Z *J Pediat* 25 468 1944
(a) 108–114 calories/kg in diet
(b) 111–118 calories/kg in diet

10 calories per kilogram on equivalent fat intakes again given either as cow s milk human milk or olive oil

Figure 173 presents the results of studies on a pair of premature twins receiving varying amounts of fat in their diet during a prolonged period of observation Both infants while on half skimmed cow s milk mixtures containing roughly 2 gm of fat per kilogram of body weight during the fore and postperiods excreted about 1 gm of the fat intake in their stools whereas during a test period of one week in which whole cow s milk mixtures contain

ing more than 4 gm of fat replaced the skimmed milk mixtures their fat excretion rose to 2 gm in one infant and over 3 gm in the other Coincidentally the weight gain was halved in one and reduced by two-thirds in the other during the test periods in comparison with the fore and postperiods

Based on this evidence it is our custom to use low fat milk mixtures in feeding premature infants especially those with birth weights of less than 1750 gm 2 to 3 gm per kilogram per day (1 0 to 1 5 gm per pound)

It is interesting to note however that no impairment of calcium and phosphorus utilization accompanies the defect in fat absorption Figure 174

FIGURE 174

Average Calcium Retention of Young Infants*

	CALCIUM BALANCE				
	Intake			Retention	
Infants	Diet†	Mg/Kg	Mg/Kg	Per Cent of Intake	Weight Gain gm/kg
Premature (1 6–2 0 kg)	H M	56	27	45 (33–51)	1 9
Premature (2 4–2 6 kg)	C M	165	114	71 (55–80)	9 4
Full Term (Lit)	C M	179‡	56†	31‡ (13–46)	8 9§

* Modified from Gordon
† Supplemented by vitamin D
‡ Jeans et al
§ Swanson

shows that in premature infants whose calcium intake increased threefold when their diets were changed from human to cow s milk their body retention of calcium rose correspondingly It is noteworthy that on equivalent intakes of calcium full term infants retain significantly less calcium as reported by Jeans and his group

The dependence of phosphorus retention on phosphorus intake is similarly shown in Figure 175 The phosphorus balance rose threefold with higher phosphorus intakes accompanying a change from human to cow s milk Here again the phosphorus balances in premature infants were of higher magnitude than those reported by Jeans for full term infants on equivalent intakes

It may be concluded that the higher contents of calcium and especially phosphorus in cow s milk, combined with effective utilization of high mineral intakes by premature infants render cow s milk more suitable from this point of view than human milk in the feeding of premature infants

FIGURF 177

Vitamin A Absorption*

No Infants	Age Days	Birth Weght gm	Fast ng	Vitam n A in Plasma 620 un ts/100 ml † 5 Hours	R se
Pren at re I fa ts					
10	28	1891	11 2	43 5	32 1
	(11 60)	(18 0–2210)	(3 4 2 9)	(8 6–83 2)	(1 9–65 2)
Fi ll Ter It fa ts					
10	19	3345	11 3	75 9	64 6
	(6–70)	(2660–4100)	(5 0–24 4)	(24 5 198 9)	(10 1 174 5)

Following test dose of 0 1 ml (6667 U S P un ts) percomorph o l per pound
† One 620 un t = 8 55 U S P un ts

Figure 178 shows that vitamin A storage in the livers of premature infants is markedly reduced Post mortem analysis of this organ gave values per gram of liver in the premature infants which were half those obtained in full term infants and on the basis of total liver content only one fifth as much Their diminished storage as indicated by decreased liver content their low plasma levels and their diminished tolerance tests indicate the need for high vitamin A supplements We use from 10 000 to 15 000 I U daily in the form of oily concentrates beginning at one week of age *

FIGURE 178

Vitamin A Content of Liver*

No Infants	Body Wt gm	Liver Wt gm	A per Gm of L ver U S P un ts	A in Whole Liver U S P un ts
Premati re Infa ts				
16	1213	53	138	7261
	(720–1830)	(45 76)	(48 138)	(2160–10 488)
Full Term I fants				
7	3310	138	303	39 994
	(2820–3985)	(82 180)	(399 266)	(32 718–47 880)

* All infants d ed less than 24 hours after b rth

The need of premature infants for vitamin C will be presented in one of my study groups At this point I merely wish for purposes of completeness to point out the key position of this vitamin in the intermediary metabolism of the aromatic amino acids phenylalanine and tyrosine When premature in fants are on a vitamin C–deficient d et of high protein content they excrete intermediary metabolites of these amino acids Administration of vitamin C abolishes the tyrosyluria It is therefore our practice to give 50 mg of

* From 1000 to 3000 I U are g ven when a water-d spers ble veh cle is used

ascorbic acid routinely to all premature infants after the first week of life especially when on high protein intakes

Because of the increased susceptibility of premature infants to hemorrhage it is our routine practice to give vitamin K to all premature infants in the form of a watery solution in dosages of 2 to 5 mg repeated if necessary

I personally have had no experience with the use of the B complex in premature infants but perhaps its role in their nutritional requirements will be taken up by Drs Bessey and Clements

On the basis of the laboratory investigations herein reported the average daily needs of premature infants are presented in Figure 179

FIGURE 179

Average Daily Needs of Premature Infants

	Per Kg	Vitamins	
Water	150 cc	A	†15 000 I U
Calories	120	D	‡3000 I U
Protein	5 gm	C	50 mg
Carbohydrates	18 gm	B Complex	?
Fat	2 gm	E	?
Calcium	160 mg		
Phosphorus	130 mg	K (Single Dose)	2–5 mg

Except vitamin K, which may be repeated
† 3000 I U if in water-dispersible vehicle
‡ 1000 I U if in water-dispersible form

The application of these methods and the results of these laboratory investigations on nutritional requirements are basic for arriving at optimal methods for feeding premature infants Some clinical applications of these laboratory studies including the relative merits of human and cow s milk in the feeding of premature infants will form the topics of study groups

This presentation is based on work done in the Pediatric Metabolism Unit of the New York Hospital–Cornell Medical Center by a group comprising Dr Harry H Gordon Dr Margaret Dann Miss Helen McNamara Miss Eleanor Marples and others The data have appeared in a series of articles published in the *American Journal of Diseases of Children* the *Journal of Clinical Investigation* *Science* and others

AROMATIC AMINO ACID METAI
IN PREMATURE INFANTS THE ROLE
AND PITUITARY A… (A

Professor LEVINE … W … ysi
creatinuria of infancy Dr … ie

discovery about ten years ago that many premature infants excreted a chromogenic substance in their urine which gave a positive Jaffe reaction but which was not creatinine

The fact that the chromogens first appeared in the urine of premature infants when their diets were changed from human milk to boiled cow s milk mixtures of higher protein content suggested that they might be intermediates of protein metabolism Positive xanthoproteic and Millon tests for the hydroxyphenyl ring gave the clue that the dietary precursors of the urinary chromogens were the aromatic amino acids phenylalanine and tyrosine

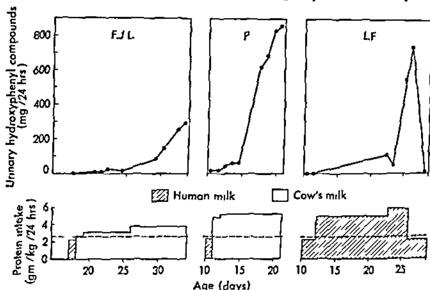


Fig 180 Relation of protein intake to appearance of urinary intermediates (Levine S Z Harvey Lect 42 308 1947)

This premise was validated by feeding these amino acids in pure form and by chemical and spectroscopic identification of the urinary compounds They were shown to be the keto and oxy derivatives of tyrosine p hydroxyphenyl pyruvic and 1 p hydroxyphenyllactic acids Methods were applied for quantitative assay

The dependence of hydroxyphenyluria on the level of protein intake is illustrated in Figure 180 Tyrosyl compounds were absent in the urine of these three premature infants when fed human milk containing less than 2 5 gm of protein per kilogram daily When the protein intake was raised to 4 0 gm or more per kilogram in the form of either human or cow s milk Millon reacting substances promptly appeared in the urine

These substances persisted in the urines for the duration of observations at

times for as long as 2 to 3 months the amounts ranging between 300 and 1400 mg of tyrosine equivalent per 24 hours for all the premature infants About 25 per cent of the tyrosine equivalent was excreted as the keto derivative *p* hydroxyphenylpyruvic acid and 75 per cent as the oxy derivative *l p* hydroxyphenyllactic acid

Full term infants receiving similar diets of boiled cow s milk of equivalent protein content did not exhibit a spontaneous tyrosyluria The defect however could be artificially produced in them by the ingestion of the amino acids in a pure state Figure 181 shows that in both premature and full term

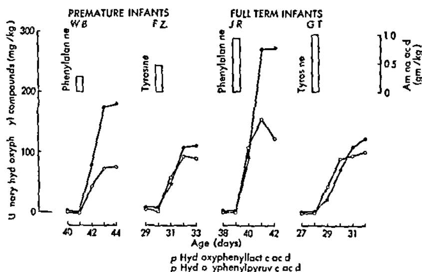


Fig 181 Ingestion of phenylalanine and tyrosine

infants a single oral dose of 1 gm per kilogram of either *dl* phenylalanine or *l* tyrosine precipitated the onset of or accentuated an already existing hydroxyphenyluria These feedings of pure amino acids not only raised the urinary output of the keto and oxy acids of tyrosine but resulted in the urinary leakage of the amino acids themselves presumably by simple overflow

Of special significance was the excretory response of phenylalanine to tyrosine ingestion and of tyrosine to phenylalanine ingestion Figure 182 shows that the urinary output of phenylalanine remained negligible even with large intakes of tyrosine whereas the output of tyrosine reached high levels with similar intakes of phenylalanine In the latter observations the voided urine often contained large numbers of insoluble crystals of *l* tyrosine

The results demonstrated that infants both premature and full term are

able to convert *dl* phenylalanine to *l* tyrosine and that this conversion is an irreversible process in vivo in the infant as had previously been shown for animals and human adults

Other amino acids including glycine methionine and tryptophane ingested in pure form and in comparable dosage failed to provoke hydroxyphenyluria

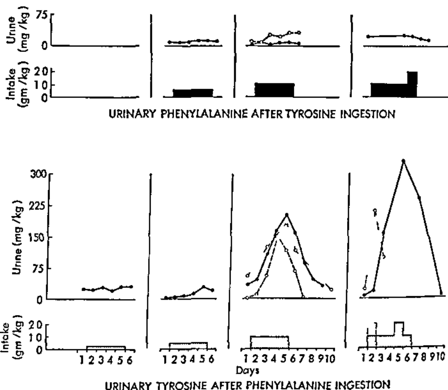


Fig 182 Urinary excretion of phenylalanine and tyrosine following ingestion of respective amino acids (Levine S Z *Harvey Lect* 42 317 1947)

Besides a high protein intake the other constant feature for the exhibition of spontaneous tyrosyluria in premature infants was a vitamin C–free diet in this instance boiled half skimmed cow s milk mixtures with vitamins A and D added It was therefore assumed that this vitamin might possess the property of eradicating the metabolic defect when present This assumption proved to be correct Typical examples are shown in Figure 183 The parenteral administration of *l* ascorbic acid in dosages of 50 mg or more abolished tyrosylurias of high magnitude within 24 to 48 hours Small daily doses of 10 to 25 mg by mouth were equally potent but the effect was somewhat delayed

Figure 184 shows the cycle of appearance of tyrosyluria with omission of vitamin C and its eradication with resumption of the vitamin on two occasions in two infants

Figure 185 demonstrates that vitamin C was as effective in abolishing the hydroxyphenylurias artificially induced in full term infants by a single oral dose of the pure amino acid *dl* phenylalanine as in the spontaneous hydroxyphenylurias of premature infants

The data establish the key position of vitamin C in aromatic amino acid metabolism in infants The therapeutic specificity of this vitamin is revealed in Figure 186 The agents listed in the table were given singly in combina

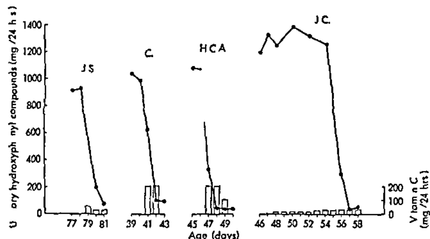


Fig 183 Eradication by vitamin C of spontaneous hydroxyphenyluria in premature infants (Levine S Z *Harvey Lect* 42 319 1947)

tion and many in summation over prolonged periods and in ample dosage without significant effect on the metabolic aberration Liver extract E 795 did produce a prompt and persistent eradication of artificially induced tyrosyluria in one full term infant but it was wholly ineffective in four premature infants and in vitamin C–deficient guinea pigs

While these observations were in progress and following their publication the important role of vitamin C in aromatic amino acid metabolism in premature and full term infants has been extended to scorbutic guinea pigs to infants and adults with latent and manifest scurvy and to in vitro experiments with liver slices homogenates and supernatants of scorbutic and nonscorbutic guinea pigs

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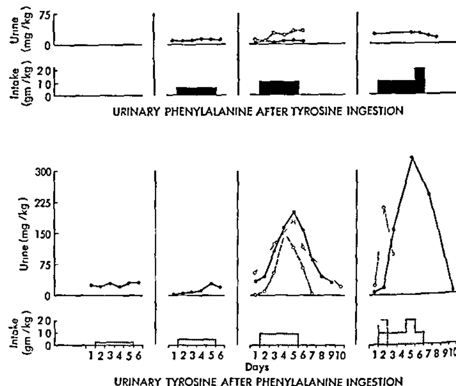


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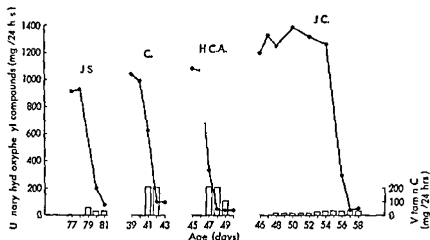


Fig 183 Erad at on by vitamin C of spontaneous hydroxyphenyluria n premature infants (Levine S Z *Har ey Lect* 42 319 1947)

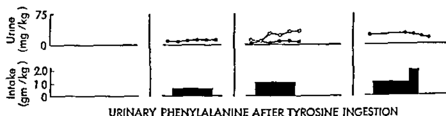
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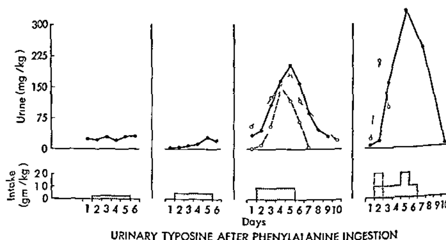


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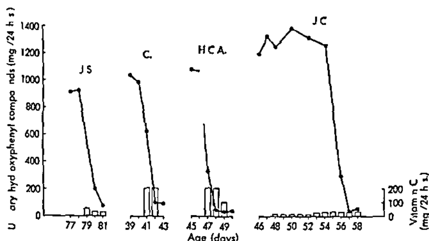


Fig 183 Eradication by vitamin C of spontaneous hydroxyphenyluria in premature infants (Levine S Z *Harvey Lect* 42 319 1947)

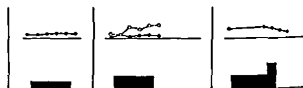
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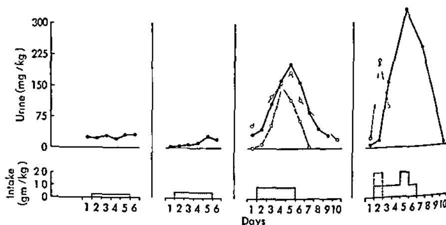


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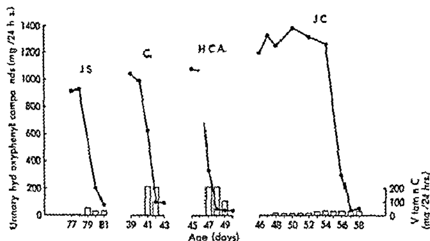


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With the advent of purified adrenocorticotropic hormone (ACTH) and

Urinary hydroxyphenyl compounds (mg /24 hrs)

RAG

800 600 400 200 0

20 22 24 26 28 30 32 34

Vitamin C (mg) 200 100 0

RMG

800 600 400 200 0

19 21 23 25 27 29 31 33 35 37 39

Age (days)

Vitamin C (mg) 200 100 0

Fig 184 Recurrent hydroxyphenyluria and eradication by vitamin C (Levine S Z *Harvey Lect* 42 320 1947)

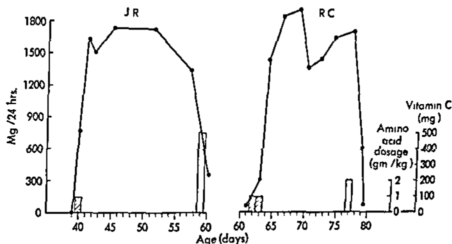


Fig 185 Eradication of artificially induced hydroxyphenyluria in full term infants by vitamin C (Levine S Z. *Harvey Lect* 42 321 1947)

cortisone (11 dehydro 17 hydroxycorticosterone) the close interrelationships between these hormones and vitamin C soon became apparent It had long been known that the ascorbic acid concentration of the adrenal cortex (especially the fasciculate and reticular zones the sites of cortisone formation) was the highest of all tissues (600 mg per 100 gm of tissue in the rat) The inverse correlation between the magnitude of cortisone secretion (as assayed by urinary corticoids) and the adrenal and urinary ascorbic acid content commonly observed in conditions of stress such as exposure to cold trauma and so on soon led to the hypothesis that ascorbic acid is needed in the synthesis of the adrenocortical hormones (cortisone) The adrenal

FIGURE 186

Effect of Other Agents on Metabolic Defect*

INEFFECTIVE

Vitamins		Other Agents
A	Thiamin	Cortate
D	Riboflavin	Creatine
α Tocopherol	Niacin	
B complex	Pyridoxine	
Ryzamin yea t	Biotin	
Crude liver extract	Choline	
	PARTIALLY EFFECTIVE	
	Liver extract E 795	
	D Isoascorbic acid	

Levine S Z *Harvey Lect* 42 323 1947

ascorbic acid depletion which promptly results from cortisone production incident to ACTH administration lends added support to this hypothesis This response is as a matter of fact so constant that it forms the basis of the standard method of assay of the potency of ACTH preparations

These interesting interrelations between ACTH the adrenocortical hormones and vitamin C instigated the present study of the effect of these hormones on the spontaneous tyrosyluria exhibited by premature infants while on diets of vitamin C–free cow s milk of high protein content Concurrent observations of the effect of these hormones on the clinical behavior body weight eosinophil counts and other metabolic features will be the subject of another panel

Nine male premature infants whose birth weights ranged from 1360 to 2240 gm were fed vitamin C–free half skimmed cow s milk mixtures soon after birth When they were on full feedings of 6 gm of protein 120 calories and 150 cc of fluid per kilogram of body weight daily and when

they were exhibiting a spontaneous tyrosyluria they were each given between the second and third week of age a 3 to 10-day course of adrenocorticotropin (ACTH) therapy in a daily dosage of 50 mg intramuscularly in divided amounts of 12 5 mg every 6 hours The total dosage ranged from 150 to 500 mg usually 350 mg over a one week period

As shown in Figure 187 ACTH in this dosage consistently abolished the tyrosyluria The qualitative disappearance of tyrosyl compounds from the

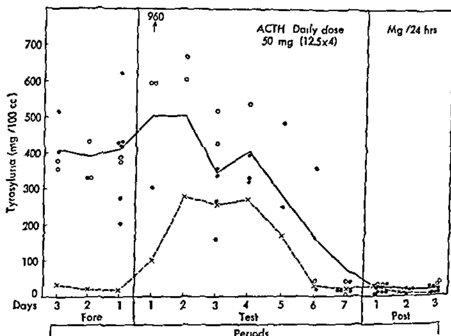


Fig 187 Effect of ACTH on tyrosyluria.

urine with ACTH therapy was confirmed by quantitative assay in 24 hour balance studies of two infants shown by open circles in Figure 187

The curative effect of ACTH was however not nearly as prompt as with vitamin C the tyrosyl compounds both *p* hydroxyphenylpyruvic and *p* hydroxyphenyllactic acids disappearing from the urine not earlier than the fifth day of treatment and in one infant being delayed until the postperiod As noted in Figure 187 eradication of tyrosyluria occurred in one infant on the fifth day of treatment in three infants on the sixth day, in four infants on the seventh day in one infant in the postperiod and none of the seven infants exhibited tyrosyluria in the first three days of the postperiod

The same daily dosage of 50 mg of ACTH administered prior to the ex

hibition of spontaneous tyrosyluria did not prevent its subsequent appearance in one infant but it did abolish the defect on the sixth day of treatment This is shown by the broken line in Figure 187 The failure of ACTH as a pre ventive agent contrasts with the prophylactic properties of vitamin C and is consistent with the delayed therapeutic action of the hormone

The curative properties of ACTH led next to a study of the minimum effec tive dose The daily dosage was halved in three infants each receiving 25 mg daily in divided amounts of 6 25 mg every 6 hours for 7 days On this total dosage of 175 mg the spontaneous tyrosyluria exhibited by the three infants disappeared on the fifth and sixth days of treatment

A further reduction in dosage to 12 5 mg per day in divided amounts of 3 125 mg every 6 hours for 4 to 7 days with total dosages ranging between 62 5 and 90 mg was equally effective in abolishing the urinary metabolites in another group of five infants

With this minimal dosage eradication of the tyrosyluria was not accom plished until the last day of treatment in one infant but it was successful in all five infants in the three day postperiod A close approximation to the total minimum effective dose was obtained in one infant who failed to respond to a total dosage of 43 75 mg administered in daily amounts of 12 5 mg for 3½ days but who responded at a later date to the same daily dosage of 12 5 mg given over a 5 day period for a total of 62 5 mg Another infant also re sponded successfully to a total dosage of 65 mg given over a 4-day period The results in the first infant are graphically portrayed in Figure 188 Tyrosyluria persisted at unchanged levels with the smaller dosage as well as with the larger dosage through the five days of treatment but a sharp drop occurred on the first day of the postperiod with virtual disappearance by the third day

The findings therefore suggest that the total minimum effective dosage of ACTH approximates 60 mg given over a period of 4 to 5 days

The therapeutic response to ACTH is apparently not only a function of the amount of hormone administered but also a function of the duration of ad ministration This conclusion is based chiefly on the results obtained in one infant and shown in Figure 189 This infant received on two separate oc casions the relatively huge amounts of 100 mg of ACTH daily for one and two days respectively without subsidence of his tyrosyluria The transient re duction in urinary intermediates which followed the larger dosage of 200 mg was succeeded by a prompt and spontaneous return to higher levels Sub sequent administration of 25 mg daily over a 7-day period for a total dosage of 175 mg resulted in the customary and prolonged eradication of tyrosyluria on the sixth day of treatment

To date the metabolic aberration has been corrected in all of the 17 premature infants male and female white and colored, who received hormone therapy for 3 or more days in a total dosage exceeding 60 mg Conversely it has not yet been possible to correct the defect with hormone therapy of

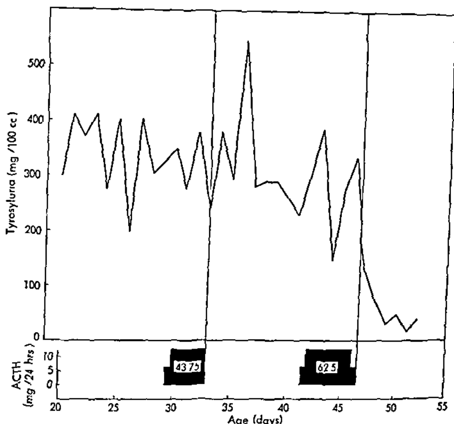


Fig 188 Effect of ACTH on tyrosyluria showing approximate minimal effective dose

less than 3 days duration irrespective of dosage up to 100 mg daily This delayed action of ACTH contrasts sharply with the prompt response to vitamin C

In 4 of the 17 infants in whom the tyrosyluria was abolished by ACTH therapy it recurred spontaneously from 10 to 18 days following cessation of treatment In the remaining 13 infants correction of the defect persisted throughout the duration of observations in the postperiods up to 25 days

The discovery of the efficacy of ACTH in abolishing the tyrosyluria of premature infants led next to a search for its mode of action It seemed

reasonable to assume that its therapeutic properties might be mediated through the stimulation of secretion of one or more of the adrenocortical steroids

Two infants were therefore given testosterone propionate by intramuscular injection in daily dosages of 5 and 10 mg over 7-day periods without effect on their tyrosyluria In both these infants ACTH produced its customary effect in one prior to testosterone therapy with subsequent recurrence of the

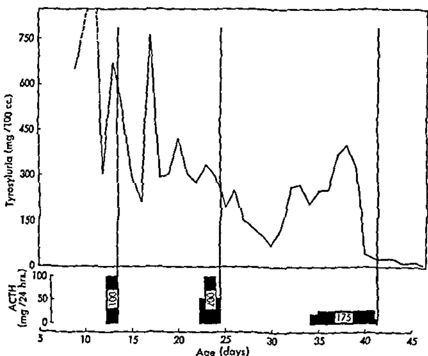


Fig 189 Effect of ACTH on tyrosyluria showing effect of duration of administration

tyrosyluria 12 days after stopping ACTH in the other following testosterone therapy after an adequate postperiod of 25 days

Another two infants exhibiting tyrosyluria were treated with desoxycorticosterone acetate in daily dosages of 2 and 3 mg for 6 and 5 days respectively without effect In one infant the tyrosyluria was precipitated by the ingestion of phenylalanine 15 days after it had been abolished by ACTH the other infant responded successfully to a course of cortisone therapy following his failure of response to DCA

Another three infants were treated with progesterone in daily dosages of 25 50 and 100 mg respectively over 7 day periods without effect on their tyrosyluria

The failure of response to testosterone progesterone and desoxycorticosterone indicates that the effectiveness of ACTH in correcting the metabolic defect in premature infants is probably not mediated through adrenocortical *stimulation of androgenic, estrogenic or electrolyte* regulating steroids This conclusion must however remain equivocal until further observations are made with larger dosage especially with testosterone and DCA

The results with cortisone remain inconclusive To date six infants have been studied in eight observations The daily dosage in these observations ranged from 25 to 100 mg the duration of periods from 3½ to 9½ days the total dosage from 280 to 762 5 mg Correction of the metabolic defect was accomplished in 3 observations on 3 infants and in all of these the minimum effect daily and total doses were 100 and 350 or more milligrams respectively *Even these relatively large amounts of cortisone were ineffective* in two infants Ingle and Sayers have both pointed out that no fixed amount of cortical hormones represents a physiological dose The metabolic responses of the organism to the same pharmacologic dosage differ markedly depending on activity environment and the functional status of the tissue cells themselves

Even if one makes the assumption on the basis of these results that the therapeutic potency *of ACTH is mediated through adrenocortical stimulation* of cortisone secretion the discrepancy between the minimum effective dose of the two hormones is striking Daily and total amounts of 12 5 and 65 mg respectively of ACTH consistently abolished the tyrosyluria of premature infants daily and total amounts of 100 and 350 mg of cortisone or more than five times the dosage of ACTH were required to abolish the defect and even these large doses of cortisone were not consistently effective *These data suggest either that intrinsic induction of cortisone secretion* by ACTH admini tration is far more potent than extrinsic administration of cortisone per se or that the potency of ACTH is mediated through an adrenocortical steroid other than cortisone

The following studies are planned or are already in progress to test th se possibilities Quantitative assay for urinary corticoids during *ACTH and* cortisone therapy may provide indirect evidence of the relative levels of cor*tisone made* available to the body by each form of hormone treatment This approach is however open to the criticism that urinary leakage of corticoids may result from simple overflow of large dosages of extrinsically administered cortisone

A second approach is to administer other adrenocortical steroids to premature infants in graduated dosage Of the 28 already isolated many are available for such a study The evidence to date suggests that if a steroid more potent than cortisone exists it will probably contain an oxygen atom in the 11 or 17 position of the cyclopentenophenanthrene ring or in both positions

Figure 190 shows the chemical constitution of the various steroids em

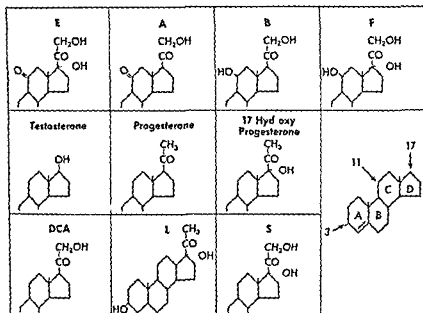


Fig 190 Chemical structure of steroids used in observations

ployed in these observations Of the four steroids the only one cortisone which has proven effective in correcting the defect in some observations contains an oxygen atom in both the 11 and 17 positions Of the three steroids which have proven ineffective in the dosages given testosterone has an oxygen atom in hydroxyl form in the 17 position in DCA and progesterone oxygen atoms are absent in both the 11 and 17 positions

Arrangements are being made to investigate the steroids listed in the figure and others as they become available to us for study

We have already had the opportunity of making single observations with Kendall s Compound A and Reichstein s Compound "L in a set of twins exhibiting tyrosyluria Compound A" differs from cortisone by the ab-

sence of an hydroxy group in position 17 compound L differs from 17 hydroxyprogesterone by the replacement of the keto by an hydroxy radical in position 3 Large daily and total doses of 100 and 700 mg in the case of each compound failed to influence the metabolic defect The question of the mechanism of action of ACTH in abolishing the tyrosyluria of premature infants must remain an open question until other steroids become available and until cortisone is used in still higher dosage

The initial impetus which prompted this investigation namely the relation if any of ACTH to vitamin C also remains unanswered Preliminary analyses of the ascorbic acid content of the plasma and the white blood cells in two premature infants during exhibition of tyrosyluria and on the day of its eradication in the course of ACTH therapy yielded constant values For the foreperiod and test period determinations as might be expected the ascorbic acid content of the plasma in both determinations in the two infants was zero the vitamin C content of the white blood cells in one infant was exceedingly low being 2 5 mg per cent and 2 6 mg per cent In the other infant the initial level was somewhat higher 16 5 mg per cent but it actually declined to 8 3 mg per cent in the test period These low values compare with average figures of 24 mg per cent for premature infants and a range between 11 and 30 mg per cent for normal infants and children who are on adequate vitamin C intakes

Further negative evidence was provided by the constant and minimal concentrations of ascorbic acid obtained in 24 hourly quantitative assays of urine throughout fore test and post periods in two balance studies each of two weeks duration

This negative evidence of the participation of ascorbic acid in the abolition of tyrosyluria induced by ACTH administration is however not conclusive since dilution may play a role in analyses of peripheral blood and urine even if ascorbic acid is liberated during adrenocortical activity

The results of these studies which are admittedly in a preliminary stage pose many more questions than they answer I would appreciate any suggestions from members of the panel with regard to what they may consider to be good methods of approach to aid in a solution of the mechanism of therapeutic potency of pituitary adrenocorticotropin in eradicating the spontaneous tyrosyluria of premature infants

Professor LINDERSTRØM LANG (Copenhagen) How much of the intake of tyrosine appears in the urine as these compounds?

Professor LEVINE (New York) In quantitative studies made following the injection of tyrosine it was found that as much as 85 per cent appeared in the urine in 96 hours

Professor LINDERSTRØM LANG (Copenhagen) Is there any indication that children given ascorbic acid grow at a faster rate?

Professor LEVINE (New York) As far as our observations went we found no difference in the rate of growth and no clinical or roentgenographic evidence of scorbutic signs—no hemorrhages hematuria or increase in capillary fragility—even though the plasma vitamin C content was zero for periods lasting two three and even four weeks Of course we hesitated to continue these studies for an indefinite period There is no question that if they had been prolonged some of the infants would have shown a diminution of growth and perhaps manifested scorbutic changes

Dr BRAESTRUP (Hellerup) Several years ago when I was studying vitamin C in newborn infants I looked for and did not find any signs which could detect what might be called preclinical scurvy To my knowledge this is the first time an effect of the lack of vitamin C has been shown before active scurvy is present

One or two of these infants were fed human milk at the beginning of the experiment In my experience it is difficult to find human milk which does not contain a considerable amount of vitamin C which makes it hard for me to explain how 15 mg of vitamin C extra daily could have any effect I wonder if the milk had been stored boiled pasteurized or treated in any way which could have influenced the ascorbic acid content?

I have the impression that your results imply that premature babies require more vitamin C than full term babies Is this justified? In Denmark we are beginning to consider whether it is justifiable to give premature babies vitamin D And we have been faced with the suggestion of giving premature babies astronomical doses of vitamin E a suggestion which I at least have difficulty in following It seems to me important that we should not give extra vitamin C to premature babies if we are not sure that this is justified According to the results of my investigations in 1936–37 the necessary dose of vitamin C is around 20 mg daily That will bring all premature and full term babies serum ascorbic acid levels up to those of breast fed children

In several instances I have followed babies for some time with about zero ascorbic values in the serum without the development of any signs whatever of scurvy

Professor LEVINE (New York) When this defect was discovered we thought it might represent a method of detecting early signs of ascorbic acid deficiency Unfortunately the plasma vitamin-C level was 0 to 0 2 mg per 100 ml for days before the defect appeared So we did not think that it represented an early stage of vitamin-C deficiency but rather a later stage

and perhaps one of the earliest signs of recovery from vitamin C deficiency Long before the plasma vitamin C level rose the defect was abolished even when the plasma levels were zero

The human milk was boiled and actual analysis showed a complete absence of vitamin C It occurred to us that perhaps some factor in the human milk other than vitamin C prevented the appearance of the defect Therefore we concentrated the human milk so that the protein intake from it alone was 6 gm per kilogram and the defect appeared indicating no such factor was present

I cannot answer the question of optimal dosage In a previous panel the same question arose with regard to vitamins A and D for prematures I do think and I think you have shown it too in your work that the higher the protein intake the more vitamin C is needed This is indicated by the work just presented a moment ago and the work of a colleague of mine Dr Dann She gave 11 premature infants vitamin C–free human milk supplying protein intakes of about 2 5 gm per kilogram She gave another 11 premature infants boiled cow s milk with protein intakes of about 5 to 6 gm per kilo gram She then gave all the babies saturation dosages of vitamin C totaling 800 mg over a period of 4 days There was a lapse of a week during which no vitamin C was given Then either 200 mg vitamin C orally or 100 mg parenterally were given and 4 hours later the plasma vitamin C level was determined In the infants receiving 2 5 gm of protein per kilogram the average vitamin-C level of the plasma was 1 9 mg per cent and in the infants receiving 5 to 6 gm of protein per kilogram intake the average vitamin C level of the plasma was 0 8 mg per cent as is shown in Figure 191 Ap parently the higher the protein intake the more vitamin C is necessary to complete the breakdown of amino acids

This has been confirmed by a recent report by Dr Jonxis in Rotterdam With paper chromatography he studied the amino acid content of the urine of infants with scurvy and he found that the amino acid output was considerably higher than normal When vitamin C was given the amino acid excretion rapidly returned to normal level He found a reciprocal relationship between protein intake and vitamin C requirements Infants receiving cow s milk (evaporated) or half skimmed cow s milk (Olac or Alacta) had a lower level of plasma ascorbic acid after a test dose of ascorbic acid than those receiving the lower protein intake supplied by human milk So perhaps in view of these data and the reduced storage of vitamin C in prematures 20 mg of ascorbic acid daily is sufficient when premature infants receive human milk but when cow s milk is used perhaps 20 mg is not sufficient

FIGURE 191

Ascorbic Acid Storage* in Premature Infants on Different Intakes of Protein

Subject†	Protein Average gm/kg day	Test Dose‡ mg	Plasma Ascorbic Acid mg 100 cc 4 hrs after test dose
		On Human Milk (Vitamin C-Free)	
1	2.6	100	1.6
2	2.7	100	1.3
3	2.7	100	1.7
4	2.7	100	3.5
5	2.8	200	2.6
6	2.7	200	1.4
7	2.7	200	1.8
8	2.7	200	1.5
9	2.6	200	1.9
10	2.7	200	2.0
11	2.6	200	1.9
			Average 1.9
		On Cow's Milk	
1	5.9	100	0.5
2	5.0	100	1.0
3	4.6	100	1.4
4	4.7	200	1.2
5	6.1	200	0.6
6	4.8	200	0.3
7	6.1	200	1.6
8	6.1	200	0.2
9	4.7	200	0.4
10	4.9	200	0.1
11	4.7	200	1.5
			Average 0.8

* Total saturation dose 800 or 900 mg l ascorbic acid over a 4 or 5-day period

† 4 males and 7 females in each group Birth weights ranged from 1.66 to 2.38 kg

‡ 5 to 7 days after saturation dose

Dr BRAESTRUP (Hellerup) Is the influence of the protein intake on the vitamin C requirement different in premature and full term babies?

Ascorbic acid has a definite renal threshold and in my experience the threshold is about 0.9 or 1 mg per 100 cc of plasma Does variation in protein intake influence the threshold?

I too have found that with human milk less ascorbic acid is needed to achieve the same serum ascorbic acid level than with cow's milk But the requirement corresponds to about 15 mg daily with human milk against 20 mg daily with cow's milk

Are there any data to show that premature babies store less vitamin C at birth than do full term babies? Dr Toverud made some studies which did suggest a relation between birth weight and ascorbic acid storage but I wonder if it was ever confirmed

Dr ELDJARN (Oslo) I should like to ask Professor Levine if ACTH or some of the other hormones or compounds mentioned have been tested in the disease of Folling—phenylpyruvic oligophrenia The metabolism of the aromatic amino acids is disturbed in this disease but as far as I remember vitamin C has been tried without any effect on the excretion of phenylpyruvic acid

Professor LEVINE (New York) We attempted to correct the metabolic defect in this disease with vitamin C and it was completely ineffective We have not seen a patient with this condition since the advent of ACTH but I think it would be well worth trying I suspect however that it would have no effect

With respect to Dr Braestrup s question about vitamin C storage I can recollect no figures but if analogies are useful all the vitamins that have been studied particularly A and B have shown a marked reduction in content in the premature organism at birth

Professor RAIHA (Helsinki) Is there any correlation between the effect of ACTH and the age of the child?

Professor LEVINE (New York) Apparently there was no correlation at all Infants who showed tyrosyluria when they weighed 1500 gm responded as well as infants whose birth weight was 2000 gm and who might have weighed 2800 gm when the studies were made Some babies incidentally still showed the defect when they weighed 3500 gm

May I point out one other thing? It has been known for a long time that infants who receive cow s milk are more susceptible to scurvy The usual explanation was that the vitamin C content of human milk was higher than that of cow s milk But if the milks are boiled more vitamin C is still needed in order to raise the plasma level when you give cow s milk than when you give human milk So I do not think that the vitamin C content of human milk is the only explanation for the increased incidence of scurvy in babies fed on cow s milk

Dr ROTHE MEYER (Copenhagen) How long does the defect persist? Does it disappear spontaneously?

Professor LEVINE (New York) When the defect is present it apparently remains continuous until vitamin C is given One such infant who was 4 months old had received all the other vitamins in succession and the defect

was still present in severe form Vitamin C then abolished the defect within 24 to 48 hours

Dr BRAESTRUP (Hellerup) Are we to conclude that we have to give the premature extra vitamin C or will it be safe to give them vitamin C in the doses which we consider normal for full term babies? I have been giving all babies including prematures 20 mg of vitamin C daily

Professor LEVINE (New York) I wish I could answer that question definitely For many years premature babies got no vitamin C and only rarely did one see scurvy manifested So that 15 mg if anything is perhaps too high a dose rather than too low

Professor BESSEY (Chicago) Here as with all the vitamins what one wants to prevent has to be decided before one can decide the requirements If one feels that it is disturbing to have this incomplete metabolism of tyrosine one should accept the dose of ascorbic acid that is necessary to eliminate it Undoubtedly however a smaller dose of ascorbic acid will prevent the development of histological scurvy What one has to select is the point where one feels safe

The histological signs of scurvy take a considerable time to develop in humans This is because structural changes occur after the chemical changes have been established and so it is not surprising that it takes weeks before clinical manifestations appear because these come after the structural changes have occurred In experimental animals the ascorbic acid level will go almost to zero many days before one sees histological changes and then it will be many days after that before one observes outright manifestations We really need to be concerned about scurvy a long time before it appears So I would disagree with Professor Levine s statement that this test is not useful in predicting early ascorbic acid deficiency

I do not think it is surprising that when one gives small doses of ascorbic acid one starts getting a response in a system like this quite a long time before the level of serum ascorbic acid rises The first demands on any nutrient of this sort are to correct a defect and not to build a concentration in tissue If one were to make histological studies in these situations one would find healing long before one could demonstrate ascorbic acid by analytical methods

Professor LEVINE (New York) I would agree completely with what Professor Bessey said The only point I was making was that the appearance of this defect occurs later than other signs of ascorbic acid deficiency such as a zero level of plasma vitamin C

The reason we were so surprised by the action of ACTH on this defect is

that theoretically at least the effect should have been the opposite There should have been an increased output of amino acids because ACTH if it *causes cortisone secretion should* have a glycogenetic effect the breaking down of protein to form glycogen moieties Therefore the ketogenetic amino acids including tyrosine might be released to increase the load to be handled by vitamin C Does that seem reasonable to you Professor Linderstrom Lang?

Professor LINDERSTRÖM LANG (Copenhagen) I do not know enough about it I only know that ACTH sometimes has an effect quite opposite to what we should expect

Professor RAIHÄ (Helsinki) *If cortisone promotes the formation of* sugar from protein then the protein when it breaks down to amino acids *would be changed to sugar and the tyrosine would disappear from the metabolism*

Professor LEVINE (New York) As I understand it there are two types of amino acids those that are glycogenetic and those that are ketogenetic Tyrosine is in the ketogenetic group and is not converted to glycogen If cortisone acts by increasing the catabolism of all amino acids then one might expect an increased output of tyrosine which would not be converted to glycogen and which therefore would have to be handled in excess of the tyrosine already present That is why I might not have expected ACTH to abolish the defect Then I asked if that were correct reasoning and the chemists did not answer

Professor BESSEY (Chicago) I do not know any more about it than *Professor Linderstrom Lang does but it seems to be that what Professor* Levine has said is right I think most of our information on whether an amino acid is glycogenetic is of such a nature that in the quantities of materials we are talking about the tyrosine might well be partially glycogenetic and we would not know it

Professor LINDERSTRÖM LANG (Copenhagen) The main thing *seems to be then that the effect of ascorbic acid and of ACTH on the system* is to reduce the deamination in general and I wonder whether there is any possibility of testing this That is why I asked about the growth Now there are so many substances that determine growth one cannot determine proteins in the babies but it may be possible that there is a reduced tendency to make proteins in these premature babies because of a disturbed balance between deamination and synthesis

Professor LEVINE (New York) I want to be sure that I understand *what is meant by deamination* Is it the elimination of the amino group?

Professor LINDERSTRÖM LANG (Copenhagen) Yes

Professor LEVINE (*New York*) Actually that does take place in the absence of ascorbic acid or ACTH because the derivatives that appear in the urine are the deaminated derivatives They are aromatic organic acids and not amino acids The amino acids themselves appear in the urine only *when the load is so great that there is a spillover* Now *is it correct* then to say that the process of deamination is not the process that is principally affected because a large proportion of the tyrosine derivatives are in the form of aromatic organic acids and not amino acids?

Professor LINDERSTRØM LANG (*Copenhagen*) *Yes* That *would* indicate that in these premature babies there is a greater deamination than usual at any rate with these compounds

Professor YLPPÖ (Helsinki) May I ask if anybody has seen any damage *from ascorbic acid? We have spoken of damage from vitamin D but nobody* has spoken of damage which might be caused by ascorbic acid For 15 years we have been giving ascorbic acid to every child from the second or third day in a dose of 25 mg twice a day We have given it together with *human milk which is usually unboiled We use this dosage* as we noticed in Finland that the seasonal curve of the ascorbic acid content of breast milk showed very low values during the winter and spring I also had a case of scurvy in a child who received only breast milk We have also hoped that *these doses of vitamin C might guard again infections* Perhaps somebody can tell us if we have been giving too large doses

Professor LEVINE (New York) I think the danger of overdosage with vitamin C is much less than the danger with vitamins D or A because vitamin C *is water soluble and is a threshold substance which is excreted in the urine* when the level in plasma reaches the threshold It is more of an economic question as to whether one is wasting vitamin C by giving large doses which are excreted in the urine

Professor WALLGREN (*Stockholm*) *In Sweden usually we do not give* extra vitamin C to premature babies until they are two or three months old I do not believe we see any more infection among our babies than among premature babies elsewhere As you know the question has been raised *whether it is possible for very young children to produce their own vitamin C* Rohmer and his co-workers in Strasbourg made experiments which they believe show that the young baby can produce vitamin C Do others of the panel give vitamin C from the first days as Professor Ylppo does?

Dr BRAESTRUP (*Hellerup*) *I give any baby not getting mother's milk* 20 to 25 mg daily A good many breast fed babies get as much as 100 mg daily in the mother's milk but I do not know of any ill effects caused by ascorbic acid

Professor LEVINE (New York) In the United States I think all babies whether on human or cow s milk get additional vitamin C *I think the* tendency is to give premature babies more vitamin C than full term babies

Professor WALLGREN (Stockholm) From the first day of life?

Professor LEVINE (New York) From the first to as late as from the *fourth day of life*

Professor SALOMONSEN (Oslo) We give all our artificially fed infants ascorbic acid from the first month of age Our prematures regardless of whether they are breast fed or bottle fed get ascorbic acid from the first day of life

Professor STEARNS (Iowa City) The artificially fed full term infants get 25 mg of vitamin C or 1 oz of orange juice and usually the latter is available They get 1 oz of orange juice until 3 months of age and then 2 oz Prematurely born infants got the same amount until Professor Levine s paper came out and then we raised it But since our discussion today since we give less than 7 gm of protein, I wonder if they really need 50 mg *I think* we may try 25 mg

Professor WALLGREN (Stockholm) It is puzzling how different the opinions and experiences are

Professor BARNETT (New York) The main reason why people began giving vitamin C earlier in the United States was the demonstration of this abnormality of protein metabolism I think also with the greater incidence of cow s milk feeding even in prematures there may be more reason to give it early in our country than in places where breast feeding is more frequent

Professor WALLGREN (Stockholm) Our premature babies get breast milk from their mothers

Professor STEARNS (Iowa City) In view of Professor Ylppo s comment that the breast milk in Finland is very low in vitamin C during the late winter months have you checked the content of breast milk here in the late winter months?

Professor WALLGREN (Stockholm) It was done in my clinic some years ago and it was a little different in the winter from what it was in the summer but it was usually sufficiently high around 5 mg per cent

Do you believe that there is some production or possibility of production of vitamin C in small babies?

Professor LEVINE (New York) *We know that this metabolic defect* persisted in small premature babies for as long as 2 months in the absence of vitamin C So if these infants were capable of producing vitamin C one would assume that the defect would have been abolished

Second vitamin-C levels remained at zero for as long as the observations

were continued without the administration of vitamin C. Of course, as Professor Bessey has pointed out, the plasma level of zero does not necessarily mean that the tissues are not getting vitamin C. But I think that the persistence of the metabolic defect and its eradication with relatively small doses of exogenous vitamin C would indicate that the infant does not produce appreciable amounts of vitamin C.

Professor WALLGREN (Stockholm): Do you usually employ ascorbic acid, or do you use the natural sources of vitamin C? In our country we give our children extracts of rose hip which we know contains a large amount of vitamin C. We use very little ascorbic acid.

Professor LEVINE (New York): I think that is true of the United States. Orange juice is used much more frequently than ascorbic acid in older children. I think that ascorbic acid is used with the prematures only because it is more difficult to give premature infants orange juice.

Professor LINDERSTRÖM-LANG (Copenhagen): Has anyone analyzed the adrenals of dead premature babies for ascorbic acid?

Dr. BRAESTRUP (Hellerup): An examination was made of the liver and, I believe, also of the adrenals by Dr. Toverud. Slightly lower values of ascorbic acid were found in premature babies.

Professor WALLGREN (Stockholm): An examination was made in Sweden of the teeth, and it is believed that very early signs of vitamin-C deficiency can be shown. And there is a team in Goteborg now investigating premature babies as to the occurrence of such changes in teeth.

A COMPARISON BETWEEN CASEIN HYDROLYSATE AND UNSPLIT CASEIN AS ADDITIONAL FOOD FOR PREMATURE INFANTS DURING THEIR FIRST WEEKS OF LIFE

Dr. MAGNUSSON* (Stockholm): The rearing of premature infants often presents some difficulties during the first few weeks of their life. This is especially true with regard to small prematures weighing, for instance, 1.5 kg or less at birth. Infants of this size have in most instances been compelled to begin their extrauterine life much too soon. A diet of breast milk alone therefore does not seem to be the ideal form of nourishment for these infants to the same extent that it is for full-term infants. The premature infant's need for so-called "Aufbaustoffen" is much greater than the full-term infant's and breast milk in many cases does not seem to cover these requirements.

On purely theoretical grounds it can be assumed that proteins are of the

* Deceased

utmost importance for the nutrition of the premature infant Not only must metabolized body proteins be replaced but proteins are necessary for growth For newborn premature infants particularly very small ones difficulties can arise when they have been deprived of their food supply from the placenta which supplied amino acids with the blood During extrauterine life nutri

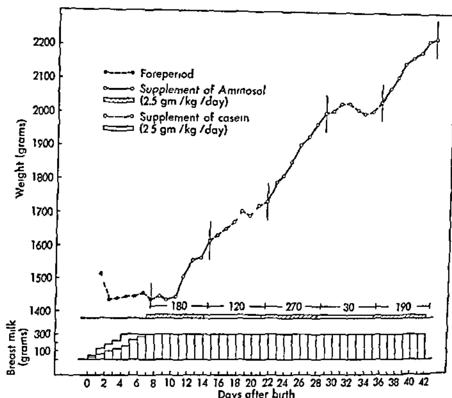


Fig 19? Weight gain in an infant born 8 weeks prematurely and in alternate periods fed breast milk and Aminosol and breast milk and casein (Magnusson J H Rev espa i pediat 4 243 1948)

tion takes place almost entirely with whole protein and there is good reason to believe that the proteolytic enzyme mechanism is defective in premature infants

It would therefore seem logical to give the premature infant a mixture containing all the essential amino acids during the first few weeks of life Since the amino acids are easily absorbed from the intestine they could be administered by mouth and given in conjunction with breast milk if the infant is receiving this This measure would seem to be one possible solution to the

problem of how to supply high-quality amino acids even to very small and very young prematures without overtaxing the digestive system

The amino acid preparation we have used is Aminosol which is an enzymatic hydrolysate of casein intended for parenteral and peroral use In order to remove any residual protein and high molecular weight peptides the hydrolyzed product is subjected to dialysis according to a method worked out by Dr Wretlind Of the mixture thus obtained the major part (76 per cent in the preoral preparation 67 per cent in the intravenous one) is composed of amino acids and the minor part of low molecular weight peptides

Two grams of Aminosol per kilogram of body weight daily is a suitable dose The powder is mixed with breast milk and administered by mouth To prematures in the lowest weight classes the amino acids are given subcutaneously at least during the first few days of life before the administration of colostrum or breast milk has been started They are then given in the form of a 3 3 per cent solution of Aminosol or a solution containing 3 3 per cent of Aminosol and 5 per cent of gluco e The preparation is well tolerated

It is of particular interest to evaluate the effects of the amino acid feedings during the earliest weeks of life In order to eliminate as far as possible the high variability which is characteristic of this stage of life it is a good plan to give the same infant alternate feedings of breast milk combined with casein and of breast milk to which Aminosol has been added over periods of equal duration 7 to 13 days for instance By comparing these periods with one another a good idea of the effects of the treatment can be gained Figure 192 illustrates this

Table 32 shows the effect we very often achieve in small prematures free from infection, during the first weeks of life In all the experimental periods each of which covered six days the total number of calories the amount of nitrogen and the salt supplied were precisely the same for all infants The difference in effect between Aminosol on the one hand and calcium paracaseinate on the other can be seen directly from the table

In order to gain a better understanding of the results of the experiments on weight gain it was considered desirable to make a comparative study of the absorption and retention of nitrogen that take place when breast milk is supplemented by calcium paracaseinate and by Aminosol I have carried out such investigations with Dr Werner in Sachs Children s Hospital between the years 1944 and 1950

All the subjects were healthy premature infants showing no signs of infection The infant was placed in a metabolic bed which consisted of an Aga couveuse having a practically constant temperature and humidity When the amount of nitrogen given was calculated allowance was made for a

of regurgitation and in the case of tube feeding for a minimum of residual food in the tube. Feces were collected in a cloth made of plastic and urine in a bottle containing 20 ml of 5 per cent sulphuric acid. The feces were normal in appearance.

Breast milk was used as the basic food in all experiments and in order to create as favorable experimental conditions as possible the amount was kept

TABLE 32

Effect of Supplementary Aminosol and Casein on Weight of Premature Infants*

Case No	Weight at birth (grams)	Day of life when the experiment began	Gain in weight (grams)			
			Experimental periods			
			1 Mother's milk + casein	2 Mother's milk + Aminosol	3 Mother's milk + casein	4 Mother's milk + Aminosol
1	1470	10	100	220	70	130
2	1500	9	90	200	60	270
3	1400	12	120	220	150	180
4	1340	12	80	160	20	130
5	1350	12	110	190	80	230
			Mother's milk + Aminosol	Mother's milk + casein	Mother's milk + Aminosol	Mother's milk + casein
6	1480	9	130	70	140	100
7	1410	10	270	120	240	150
8	1390	11	240	125	290	50
9	1510	9	140	70	110	120
10	1500	10	170	120	270	80

* *Magnusson J H Rev espan pediat 4 244 1948*

fairly low. In some instances we gave a constant amount of breast milk per kilogram of body weight daily and in others the total amount of breast milk given to an infant was kept constant for the duration of the experiment.

The results will be seen in Tables 33 to 37 inclusive. Table 33 shows the nitrogen metabolism in six premature infants during the first weeks of life. In five of them as an addition to the breast milk calcium paracaseinate and Aminosol respectively were given in quantities corresponding to 250 mg

of nitrogen per kilogram of body weight daily. Case VI was included as a control. Each observation period lasted for three days and was preceded by an adaptation period of the same length during which the infant received exactly the same food as during the observation period. The values in the table with the exception of those for the amount of urine are calculated per kilogram of body weight and comprise the total values for these three days.

TABLE 33

Effect of Supplementary Aminosol and Casein on Weight and Nitrogen Retention in Premature Infants

Case	Diet in period of study	N in food (mg)	Absorbed N mg	Absorbed N Per cent of N in food	Retained N mg	Retained N Per cent of N in food	Retained N Per cent of absorbed N	Increase in weight (gm)	Urine (ml)	Ave age weight (gm)	Age (days)
I	B m + a	1551	1300	85	918	59	0	95	506	2200	15 17
	B m + c	17 6	1632	92	8 4	49	54	23	89	2160	21 23
	Br m + a	1467	1323	92	841	57	64	39	495	255	2 29
II	Br m + a	2001	1782	89	1265	63	1	67	650	2009	16 18
	Br m + c	17/5	1521	86	843	48	55	33	8 3	2242	21 23
III	Br m + c	1600	1230	90	647	41	53	7	616	1493	5
	B m + a	2217	1932	85	1439	65	76	88	428	1693	12 14
IV	Br m + a	1572	1413	90	99	63	71	35	371	2 5	37 39
	Br m + c	1451	1265	88	669	46	53	17	524	2643	44 46
V	Br m + a	2034	1779	8	1332	68	78	109	505	160	14 16
	Br m + c	2040	1777	88	936	46	53	38	672	1828	20 22
	Br m + a	1735	15 6	91	953	54	61	66	594	2101	27 29
VI	Br m	1172	951	77	675	58	71	46	11	2150	12 15
	Br m	1129	903	77	656	58	3	40	745	2233	15 1

* The following abbreviations are used in Tables 33 37 Br m = breast milk c = calcium paracaseinate a = Aminosol

It will be seen from this table that the absorption of casein N appears to take place as effectively as that of Aminosol N. Casein N and Aminosol N however behave differently since casein N is excreted in considerable amounts and the Aminosol N is retained.

The difference in the weight increases noted in the Aminosol periods and the casein periods is consistent. The Aminosol period shows greater increases. The increase in weight is reflected in the quantity of urine which is constantly lower during the Aminosol periods. Because of individual varia

TABLE 34

Effect of Supplementary Aminosol and Casein on Weight and Nitrogen Retention in a Pair of Premature Twins

Case	Diet in period of study	Age (days)	N in food (mg)	Feces N (mg)	Absorbed N		Urine N (mg)	Retained N			Increase in weight (gm)	Average weight (gm)
					mg	Per cent of N in food		mg	Per cent of N in food	Per cent of absorbed N		
VII 470/49	Br m	16–18	1800	529	1271	71	499	772	43	61	60	1763
	Br m + c	25–27	3358	572	2786	83	1016	1770	53	64	70	1980
	Br m + a	32–34	3549	465	3084	87	774	2310	65	75	100	2193
	Br m + c	39–41	3354	405	2949	98	1472	1447	44	50	20	2300
VIII 471/49	Br m	15–17	1728	372	1356	78	573	783	45	58	55	1865
	Br m + c	25–27	3236	571	2665	82	1091	1574	49	59	40	2023
	Br m + a	32–34	2984	441	2543	85	684	1839	62	73	110	2213
	Br m + c	39–41	2888	306	2582	99	1186	1396	48	54	40	2303

tions it is obvious that only observation periods from the same case should be compared with one another

Table 34 shows the results in a pair of twins born six weeks before term with birth weights of 1810 gm and 1930 gm During the entire experiment 300 gm of breast milk daily were given as basic food The experiment was carried out in the same manner as those shown in Table 33 The adaptation periods were slightly longer however and the amount of nitrogen supplied in the form of calcium paracaseinate or Aminosol was a little higher

TABLE 35

Diet in period of study	Age (days)	N in food (mg)	Absorbed N mg	Absorbed N Per cent of N in food	Urine N (mg)	Retained N mg	Retained N Per cent of N in food	Retained N Per cent of absorbed N	Increase in weight (gm)	Case
Br m + c	16–18	1749 (945 + 804)	1558	89	685	8 3	50	56	39	I
Br m + a	22 24	1672 (868 + 804)	1546	92	662	884	53	57	64	456/47
Br m + c	28–30	1640 (836 + 804)	149	91	893	602	37	40	20	
Br m + a	34–36	1624 (836 + 804)	14 4	92	893	601	37	40	20	
Br m + c	19–22	1560	1359	87	7 0	590	38	43	18	II
Br m + a	26–29	1537	1349	88	796	554	36	41	21	5 50
Br m	12–15	803	673	84	261	406	51	60	19	III
Br m + c	16–19	1575	1357	86	657	705	45	52	15	69 50
Br m + a	23–26	1557	130	84	756	549	35	42	7	
Br m + c	30–33	1536	1412	92	1010	410	2	29	4	
Br m + a	35 38	1540	13 1	86	913	433	28	33	7	

The values in Table 34 correspond to the total values for three days and were *not* calculated per kilogram of body weight The figures are very much the same in both cases The results shown in Table 33 are confirmed here The amount of nitrogen retained and the gain in weight are greatest during the Aminosol periods

Table 35 shows the results obtained in three full term infants weighing over 3 kg at birth During the experiment 500 gm of breast milk were given each day as the basic diet The experiments were carried out in the same way as those shown in Tables 33 and 34 The amount of supplementary

nitrogen administered in the form of calcium paracaseinate or Aminosol 804 mg in case I corresponds to a little over 1 5 gm of protein per kilogram of body weight daily The values given in this table are the total values for three days for each kilogram of body weight

There is no difference in nitrogen retention between the periods when the infants received calcium paracaseinate and those when they were given Aminosol

TABLE 36

Diet in period of study	Age (days)	N in food (mg)	Absorbed N		Retained N		
			mg	Per cent of N in food	mg	Per cent of N in food	Per cent of ab sorbed N
1 Br m	9–11	1800	1325	74	+ 725	40	55
2x Br m + c	12–14	1800 + 2985			−1614		
2y Br m	15–17	1800			− 351		
Average of 2x and 2y		3292	2632	80	+ 632	19	24
3x Br m + a	20–22	1800 + 2985			+2455		
3y Br m	23 25	1800			+ 300		
Average of 3x and 3y		3292	2870	87	+1378	42	48
4x Br m + c	28 30	1800 + 2985			+1619		
4y Br m	31–31	1800			− 149		
Average of 4x and 4y		3292	2816	86	+ 735	22	26

In Table 36 we find the nitrogen metabolism in a premature infant born 4 weeks before term and weighing 1850 gm at birth At 9 days of age when the experiment was begun the infant weighed 1770 gm

The nitrogen metabolism on breast milk was first studied Thereafter relatively large supplementary amounts of calcium paracaseinate and Aminosol respectively were given during the observation periods (i e the periods 2x 3x and 4x) each of which was of three days duration The supplementary amount corresponds to 995 mg of nitrogen daily and thus to 2985 mg of nitrogen per observation period The figures in the table show

the total values for three days The periods 2y 3y and 4y correspond to the three-day period immediately following the respective observation periods Breast milk alone was given during each y period These were included because the change in the nitrogen balance which was brought about by the large supplement of nitrogen during the x period could not be completed during the three-day observation period The effect of the additional nitrogen is therefore best studied as an average of x and y

It will be seen from the table that the absorption of casein N appears to take place as effectively as that of the Aminosol N even when the doses are

TABLE 37

Diet in period of study	Age (days)	N in food (mg)	Absorbed N		Retained N		
			mg	Per cent of N in food	mg	Per cent of N in food	Per cent of absorbed N
1 Br m	11–13	2400	1891	79	1280	53	62
2x Br m + c	14–16	2400 + 3864			3740		
2y Br m	17 19	2400			638		
Average of 2x and 2y		4332	3700	84	2192	51	58
3x Br m + a	20–22	2400 + 3528			2991		
3y Br m	23–25	2400			1119		
Average of 3x and 3y		4164	3489	84	2055	49	59

large Here also however Aminosol N is retained in much larger amounts than casein N

Table 37 shows the nitrogen metabolism in a full term infant given large supplementary doses of calcium paracaseinate and Aminosol respectively together with ordinary breast milk The infant s weight at birth was 3420 gm and its age at the start of the experiment 11 days The method was exactly the same as in the case shown in Table 36

In the full term infant there is no marked difference in the respective amounts of Aminosol N and casein N retained

We have now been using casein hydrolysate for 8 years as a routine additional diet for premature infants and our experience has been consistently such as to suggest that it is more advantageous to supplement breast milk with

a casein hydrolysate than with casein It seems to me that it is possible in this way to provide valuable additional nourishment without causing any functional strain

To summarize The addition of a suitable amount of an enzymatically digested and dialyzed hydrolysate of casein to breast milk makes an excellent food for premature infants It is especially suitable for very small prematures during the first weeks of their life

PEPTIC AND TRYPTIC CAPACITY OF THE DIGESTIVE GLANDS IN NEWBORNS A COMPARISON BETWEEN PREMATURE AND FULL TERM INFANTS

Dr WERNER (Stockholm) In fresh post mortem material the proteinase producing digestive glands in premature and full term infants have been examined In 70 infants (47 premature) the gastric mucosa and in 41 infants (26 premature) the exocrine pancreatic tissue were examined histologically as to the maturity of their enzyme producing cells Chemical determinations of the proteinase content were made in 30 infants (22 premature) as regards the gastric mucosa and in 24 (16 premature) infants as regards the exocrine tissue of the pancreas

I am quite aware that post mortem examination of the enzyme producing glands gives no exact information concerning the glands in vivo Since however the investigation was made as a comparative study with equal treatment of specimens from premature and full term infants the factors influencing the results ought to have been the same in both groups An investigation of this kind made on post mortem material must necessarily deal with the weaker part of the group to be studied but in this discussion it is just the weaker patients that are of interest In a clinical investigation on the contrary there might be a selection of stronger and healthier patients

Ideal post mortem material would consist only of infants who died because of instrumental intervention during labor Since it was impossible to obtain more than a few such patients other material had to be used Cases have been selected in which the cause of death might not be expected to influence the amount of enzymes in the glands Furthermore premature infants who died because of a cause other than debility were always compared with full term infants in whom the cause of death was the same As is seen in Table 38 most of the prematures died either from debility alone or from debility and pulmonary atelectasis The infants were all in the first week of life and most of them lived for less than 48 hours This is a detail to be stressed because the situation may change rather soon as the infant grows older

In the histological work the pepsinogen granules in the gastric mucosa and the zymogen granules in the pancreas have been taken as a criterion of the functional maturity of the tissue This should be justified since Bowie in 1936 demonstrated an intimate relationship between the pepsinogen granules and pepsin Harper and Mackay in 1948 did the same for the zymogen granules of the pancreas and the pancreatic enzymes With the stain used the pepsinogen granules take a dark violet color in striking contrast to the rest of the tissue

TABLE 38
Gastric Material Studied Histologically Causes of Death of the Infants

Cause of death	Number of cases	
	Premature†	Full term
Congenital debility (only)	17	—
Intervention during parturition	3	3
Pulmonary atelectasis	12	3
Intracranial hemorrhage	8	3
Intracranial hemorrhage + pulmonary atelectasis	4	5
Bronchopneumonia	1	1
Congenital heart disease	0	3
Intrauterine asphyxia	1	3
Erythroblastosis fetalis	0	1
Suprarenal apoplexy	1	1
	47	23
	Total [illegible]0	

Werner B *Acta paediat* 3[illegible](suppl 6) [illegible]0 1948
† Birth weight < 500 gm

In a human adult the pepsinogen granules occupy three fourths of the cross section of the mucous membrane and in a four months-old infant the border of granules is still wide In the full term newborn infant there is only a narrow border of granules in the basal part of the mucosa but it is nevertheless quite conspicuous (Fig 193)

In a small premature infant no border of granules can be seen (Fig 194) The general picture of the mucosa is one of immaturity The pits are wide and the glandular tubules are short and widely spaced The mucous cells at the surface are well developed even in the smallest premature infants

In the premature group over 2000 gm there is no considerable increase in the amount of granules in the chief cells The structure of the mucosa how

ever has a more mature appearance and because of the increased number of glands the total amount of granules is greater than in the lower weight groups

Because we have found a marked border of granules in the full term infant and very few granules in even the rather heavy prematures we believe there is an acceleration in the formation of granules just before term This is in accordance with Miller s results concerning the amount of hydrochloric acid in the stomachs of newborns In 106 cases he found the gastric acidity to be low in premature as compared with full term newborns

In 30 cases the peptic activity was estimated in acid extracts of excised samples of the mucosa with known surface area For the analyses Anson s

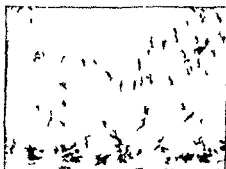


Fig 193 Case No 33 Birth weight 3680 gm full term gastric mucosa a marked border of pepsinogen granules (Werner B *Acta paediat* 35[suppl 6] 32E 1948)

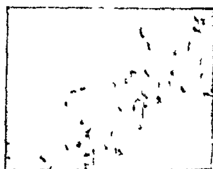


Fig 194 Case No 49 Birth weight, 1020 gm premature gastric mucosa no pepsinogen granules discernible (Werner B *Acta paediat* 35 [suppl 6] 32L, 1948)

colorimetric method was used with slight modifications because of the crudity of the extracts This method was well suited for this work since the results are not influenced by the presence of peptidases in the digestion mixture

The results are shown in Figure 195 There is a considerable dispersion in the premature group with both rather high and very low values No premature infant however shows a value that comes up to the lowest value for the full term infants

The pancreatic tissue was prepared histologically in the same way as the gastric mucosa

In a full term newborn infant the zymogen granules are almost as abundant as in older children (Fig 196) In premature infants under 1500 gm the granules are practically absent (Fig 197)

In 24 cases the proteolytic activity was determined in enterokinase activated

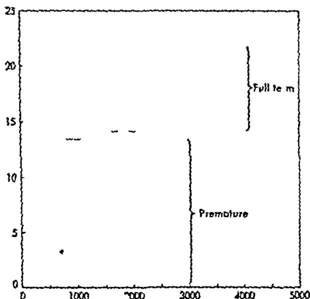


Fig 195 Diagram of the relation between the values for the peptic activity of the gastric mucosa from the 30 infants examined Cases arranged according to weight at birth Abscissa weight of the infants in grams Ordinate peptic activity expressed as milliequivalents × 10⁻⁴ tyrosine per cubic centimeter of trichloroacetic acid filtrate (Werner B *Acta paediat* 35[suppl 6] 42 1948)

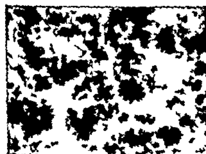


Fig 196 Case No 10 Birth weight 3610 gm full term pancreas rather rich in zymogen granules (Werner B *Acta paediat* 35[suppl 6] 60 1948)

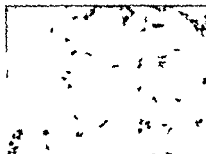


Fig 197 Case No 4 Birth weight 14 0 gm premature pancreas without zymogen granules (Werner B *Acta paediat* 35[suppl 6] 60 1948)

glycerol extracts of defatted dried pancreas powder The results are shown in Figure 198

The values for the prematures are low as compared with those for the full term infants A single deviating case with a higher value indicates that a larger series might have shown the prematures somewhat better off It must however be emphasized that there are individual cases with extremely low values

Because of the marked difference in the maturity of the cells of the two groups and the great difference between the lowest premature values and the

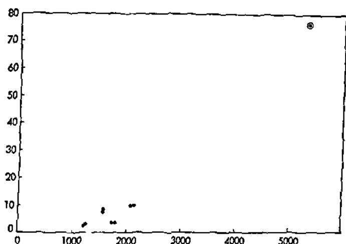


Fig 198 Diagram of the relation between the values for the proteolytic activity of pancreas dry powder from the 24 infants examined Cases arranged according to weight at birth Abscissa weight of the infants in grams Ordinate proteolytic activity expressed as milliequivalents × 10^{-4} tyrosine per cubic centimeter of trichloroactic acid filtrate (O = proteolytic activity of dry powder from pig pancreas) (Werner B *Acta paediat* 35(suppl 6) 58 1948)

mean full term value as regards enzymatic activity it should be reasonable to assume a difference in the ability of full term and premature infants to digest protein

In a paper published this year from Freudenberg s Clinic in Basel Geschwind has in post mortem material investigated the occurrence of lipase amylase and proteinase in the pancreatic glands of 56 newborn premature and full term infants In that work reference was made to the total weight of the pancreatic gland and to the requirement of the infant for protein The conclusion was drawn that the premature infant is severely handicapped in comparison with the full term one as regards capacity to digest protein

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Dr WRETLIND (Stockholm) Aminosol* is prepared from bovine casein by hydrolysis with trypsin and erepsin In order to free this digest from all unhydrolyzed proteins and high molecular weight peptide the hydrolysate is dialyzed In this way a product containing chiefly free amino

TABLE 39*†

Preparation	Total nitrogen mg	Amino nitrogen mg	Amino nitrogen / Total nitrogen
Aminosol for peroral use without acid hydrolysis	6 02	3 2	0 54
Aminosol for peroral use after acid hydrolysis	5 89	4 17	0 1
Aminosol for intravenous injection without acid hydrolysis	4 [illegible]	2 39	0 50
Aminosol for intravenous injection after acid hydrolysis	4 67	3 46	0 75

Wretlind, K A J *Acta phys ol scandinav* 13 48 1947
† 5 00 gm of Aminosol for peroral administration were dissolved and made up to 100 ml with distilled water 1 ml of this solution was taken for the analyses This implies that the peroral preparation contains 12 per cent nitrogen For the determinations on Aminosol for intravenous use 1 ml of the commercial 3 3 per cent solution was used

acids and a smaller amount of low molecular weight dialysable peptides is obtained The nitrogen and amino nitrogen analysis of this preparation is given in Table 39 In the Aminosol for oral use the relation between the amino nitrogen and total nitrogen increases during acid hydrolysis from 0 54 to 0 71 This means that in Aminosol 76 per cent of the nitrogen is in the form of free amino acids The other 24 per cent is peptides The preparation of Aminosol for intravenous use on the other hand is composed of 67 per cent free amino acids and 33 per cent low molecular peptides For the identification of these free amino acids paper chromatography was used in many of the tests These were performed by a modification of the method

* Aminosol is prepared by Vitrum Stockholm

of Consden Gordon and Martin (*Biochem J* 38 224 1944) revised by Edman (*Arkiv for kemi mineralogi och geologi* 22A No 3 1945) The following amino acids were identified tyrosine proline trytophane valine methionine phenylalanine, isoleucine leucine aspartic and glutamic acids Lysine and arginine were identified by paper chromatography after their isolation by electrodialysis Histidine was first precipitated as the silver salt and then identified by chemical methods Thus most of the amino acids have been shown to appear in free form in Aminosol

On a diet containing only the nine synthetic amino acids essential for growth (Rose *Physiol Rev* 18 109 1938) rats grow fairly well (Wretlind *Acta physiol scandinav* 17 Suppl 59 1949) In Table 40 the amino acids are

TABLE 40

Grouping of Amino Acids into Those Essential for Growth Essential for Optimal Growth and Nonessential*

Amino acids essential for growth	Amino acids essential for optimal growth	Nonessential amino acids
Histidine	Arginine	Alanine
Isoleucine	*Glutamic acid*	*Aspartic acid*
Leucine		Citrulline
Lysine		Cystine
Methionine		Glycine
Phenylalanine		Hydroxyproline
Threonine		Norleucine
Tryptophane		Proline
Valine		Serine
		Tyrosine

* Wretlind K A J *Acta physiol scandinav* 17(suppl 59) 14 1949

ranged in three groups with respect to their growth effects in rats If one of the amino acids in the first column is removed from the diet the animals will lose weight and eventually die The amino acids listed in this column are all, except histidine also essential for human beings If one of the amino acids in the second column is lacking in the diet a decreased gain in weight will result The third column contains the nonessential amino acids which have no effect whatever on growth when amino acids from the two other groups are present in sufficient concentrations In Aminosol all the amino acids belonging to the first two groups are present in free form as well as the nonessential amino acids alanine aspartic acid proline serine and tyrosine The concentrations of free cystine glycine and hydroxyproline in Aminosol

are too low to be determined by paper chromatography Citrulline and norleucine are not present in casein Table 41 lists the concentration of essential amino acids in Aminosol These were determined by microbiological methods and are about the same as in casein

It is not only necessary to have all the essential amino acids present but it is also necessary that they be present in the right proportions The best way to determine that the proportions are right is with feeding experiments With Aminosol young rats grow as well as they do with corresponding amounts of casein they retain nitrogen and they show an increase in body protein

A protein hydrolysate like Aminosol requires no further hydrolysis by the enzymes of the intestinal tract and is very rapidly absorbed Ten milliliters

TABLE 41

Concentration of Essential Amino Acids in Aminosol

Amino acid	Per cent
Arginine	2
Histidine	1 8
Isoleucine	3 2
Leucine	6 0
Lysine	4 4
Methionine	2 3
Phenylalanine	4 1
Threonine	2 1
Tryptophane	1 1
Valine	3 6

of a 3 3 per cent Aminosol solution were injected into an isolated 18-cm loop of the small intestine of a dog beginning 20 cm below the entrance of the pancreatic duct The injected amino acids were completely absorbed within 30 minutes

Such an amino acid preparation has a rather high buffering capacity Between pH 8 and 5 the buffer action is comparatively small but below pH 5 it increases markedly To decrease the pH from 7 to 3 it is necessary to add 23 5 ml of 0 1 *N* HCl to 1 gm of Aminosol This buffering activity of amino acid preparations has also been used in the treatment of peptic ulcers

Goldberg and Wretlind (*Acta physiol scandinav* 14 19 1947) have investigated the toxicity of Aminosol In order to kill a rabbit with a single oral dose 30 to 40 ml of a solution containing 20 to 40 per cent Aminosol had to be given From these results it was calculated that the lethal oral single

dose is about 12 5 to 15 gm per kilogram The lethal single intravenous dose is between 6 5 to 17 3 gm per kilogram depending on the animal since rabbits were more sensitive than guinea pigs This corresponds in a 70 kg man to a single dose of 445 to 1210 gm of amino acids given intravenously

Nor do these dialyzed preparations contain any anaphylactogenic properties It is impossible to sensitize or produce anaphylactic reactions in guinea pigs with Aminosol With an enzymatic casein hydrolysate which has not been dialyzed it is however possible both to sensitize and produce anaphylactic shock

Dr BRAESTRUP (Hellerup) *What is the total chloride content of the amino acid preparation?*

Dr WRETLIND (Stockholm) It does not contain any chloride The only salt it contains is sodium phosphate In order to maintain the optimal pH of 7 to 8 for the enzymatic hydrolysis sodium hydroxide has to be added This forms sodium phosphate with the phosphate ions liberated from the casein

Dr ROTHE MEYER (Copenhagen) Do you add hydrochloric acid for the hydrolysis?

Dr WRETLIND (Stockholm) No The protein is hydrolyzed by trypsin and erepsin at a pH of 7 to 8 Incidentally that pH can be obtained with ammonium hydroxide if you do not want sodium ions in the preparation

Professor LEVINE (New York) In addition to protein what fats and *carbohydrates were in the feedings given by Dr Magnusson?*

Dr MAGNUSSON (Stockholm) Breast milk was given as the basic food and pure Aminosol in powder form or casein as a rule in the form of calcium paracaseinate was the only addition

Professor LEVINE (New York) What is the total amount of protein fat and carbohydrate per kilogram of body weight that the infant receives when fed breast milk with the addition of Aminosol or calcium paracaseinate?

Dr MAGNUSSON (Stockholm) Our investigations are not yet com pleted and I am therefore unable to give any exact figures at present

Professor LEVINE (New York) It seems to me that the preparation *that Dr Magnusson used differs from human milk primarily in that it has* more nitrogen The fat content is perhaps higher and the carbohydrate con tent may be somewhat lower than in straight cow s milk The observations of body weight gain and nitrogen retention certainly establish that the premature infant grows exceedingly well and retains nitrogen when given this combina tion Other observations show that gain in weight and retention of nitrogen occur very well when the nitrogen intake is increased by using cow s milk The critical observation would seem to be to compare the gain in weight and

nitrogen retention and the change in general condition of premature infants when given human milk plus casein as one study human milk plus casein hydrolysate as another study (those two have already been done and show casein hydrolysate is apparently more effective than unsplit casein) evap orated cow s milk with the same amount of fat as in human milk plus casein hydrolysate as a third study and half skimmed cow s milk with less fat plus casein hydrolysate and the calories made up with carbohydrate as a fourth study Dr Magnusson has established the validity of his type of feeding and other observers have established the validity of using cow s milk in premature infants The question is which is better if either

Dr Magnusson emphasizes the percentage nitrogen retention in these bal ance observations However the absolute nitrogen retention is far greater when added protein is given in the form of either casein or Aminosol Thus more nitrogen is retained with the higher intake That seems to me to be the important fact for growth in a premature infant

Dr Werner s work certainly proves that in post mortem examination there is a definite correlation between the histologic appearance and chemical ac tivity of the gastric and pancreatic glands Dancis recently fed protein hydrolysate and unsplit casein to two groups of living premature infants He studied peptic activity by duodenal drainage and found that in both in stances the drainage fluid produced good liquefaction of the material fed He also found the gain in weight to be the same in both groups of infants

I did not realise that glutamic acid was a partially essential amino acid for rats as Dr Wretlind pointed out Has it been shown to be partially essential for human beings?

Dr MAGNUSSON (Stockholm) I think an investigation of the kind proposed by Professor Levine would be necessary to clear up the different points mentioned It would be very difficult to carry out here in Stockholm however because our mothers are convinced that the smaller the baby is the more necessary it is to feed it breast milk If a collaborative effort were undertaken I should be glad to take part in it

Dr WERNER (Stockholm) Macky and Dancis studied the enzyme con centration in the so-called basal secretion Do you think that any conclu sions about the total capacity of the pancreatic gland can be drawn from basal secretion studies Professor Levine?

Professor LEVINE (New York) I should like you to answer that

Dr WERNER (Stockholm) They found that the enzyme concentration in the juice obtained during basal secretion was the same in premature and full term infants There may however be an important difference between these two groups of infants in the total capacity of the pancreatic gland

Even if the enzyme producing capacity of the pancreas is small it may nevertheless suffice to saturate the fluid produced during the basal secretion We have just begun to study the proteolytic capacity of the pancreas with the help of secretin and pancreozymin After collecting samples of basal secretion juice secretin is given to wash out the enzyme accumulated in the ducts After that the protease in the enzyme producing cells is released by repeated injections of pancreozymin Up to now we have only two comparable cases one premature and one full term infant and of course conclusions cannot be drawn from two cases In these two cases however there was no correlation between the amount of enzyme in the basal secretion and the amount of enzyme produced later during the test period

Professor LEVINE (New York) I think that is a very satisfactory answer What about the weight gains?

Dr WERNER (Stockholm) I have nothing particular to say about the weight gains

In the last few weeks I have been trying to supplement my studies on post mortem material with clinical observations on the pancreatic function in premature infants A test meal of casein was given and the amino nitrogen level in the blood followed during the next four hours The next day a test meal of Aminosol was given to the same infants for comparison Between a group of four full term infants and a group of five premature infants all with birth weights below 1500 gm I found a marked difference in the amino nitrogen level following casein feeding but practically none after Aminosol feeding The sources of error in this comparison may be big enough to make the difference open to question The premature liver is comparatively large and the absorption area in the intestine small Yet why should the difference disappear with a test meal of protein hydrolysate? The amino acid estimations were made on whole blood colorimetrically and ought to be checked for instance by a gasometric method preferably on plasma What do you think about this kind of test Professor Levine?

Professor LEVINE (New York) I think it desirable to extend these investigations If the results are significantly different they may be of importance I should like to hear the opinion of the biochemists regarding the method

Dr WERNER (Stockholm) Do you think that in principle a test like this can be used in comparing infants in different stages of development?

Professor LEVINE (New York) From the point of view of the premature infant s capacity to absorb amino acids if one demonstrated good absorption with Aminosol and not as good absorption with unsplit casein that would be additional evidence in favor of the use of Aminosol One might

not however be able to make comparisons between the absorption rates in premature and full term infants because of basic differences in configuration as you have already implied

Dr WRETLIND (Stockholm) In answer to Professor Levine s other question a diet including all the amino acids except glutamic acid will produce a gain in weight of growing rats but the gain is less than if glutamic acid is included in the diet This has not yet been shown to be true in human beings It is very expensive to perform such experiments because the subject has to be kept on a diet of pure amino acids for a long time

Professor YLPPÖ (Helsinki) Was the unsplit protein given in the form of calcium caseinate or pure casein?

Dr WERNER (Stockholm) Calcium caseinate

Professor YLPPÖ (Helsinki) There is a very great difference between different forms of casein For example it is very difficult to dissolve pure casein and calcium caseinate can be dissolved and digested only if given in the form of a very fine powder

Dr WERNER (Stockholm) We used the fine powder

Professor YLPPÖ (Helsinki) For many years we have used these amino acids and other preparations and have always noticed an increase in weight However the idea of always obtaining the same increase of weight after birth may be an ideal but it is not possible or physiological for every child

Professor BARNETT (New York) In Dr Werner s demonstration there is evidence that there are certain functions in the premature infant which seem to be fairly independent of the event of birth In a rather indirect way this seems to support the concept that the premature baby after birth is not as different from the fetus as one might suppose from the fact that it is in such a different environment Further support for this concept is found in the development of the glomerulus which tends to reach completion histologically at around a body weight of 2500 to 3000 gm independent of when birth occurs The fact that an infant s kidney has been functioning for 100 days of what should have been intrauterine life does not seem to have much influence on the development of the kidney One might also have expected that as soon as the premature baby starts eating the rate of development of the stomach and pancreas histologically and physiologically would accelerate function but apparently it does not. Thus many of the functions of the premature infant which are used postnatally continue to develop as if they were not being used. This is of course a highly speculative argument

Dr ROTHE MEYER (Copenhagen) It seems that there are essentially two different schools of thought in feeding premature infants today O gives as little food as possible and the other tries to imitate the developm

the premature infant as it would have occurred in utero We have tried to obtain the same amount of weight increase as there would have been in fetal life and have produced daily increases of between 25 and 30 gm without apparent harm to the premature We have tried feeding half skimmed acidified cow s milk mixtures as Professor Levine has and also tried human milk supplemented with amino acids We did not have good results because the Danish preparations are not effective During the last year we have used human milk with a casein supplement and have had I think as good results as before

Professor LEVINE (New York) May I ask Dr Salomonsen what method is used in Norway to feed premature babies?

Professor SALOMONSEN (Oslo) In recent years we have been using *Aminosol as a supplement to human milk*

I was surprised at the frequent occurrence of rickets in premature infants in Sweden Rickets is not so common in Norway and I wonder if this difference can be related to the fact that although it is used in my clinic Aminosol is not as generally used in Norway as it is I suppose in Sweden Can an increased growth rate be related to a higher incidence of rickets in Sweden?

Professor WALLGREN (Stockholm) The high incidence of rickets in premature infants in Sweden is a thing of the past With the massive vitamin D therapy in use now it is impossible to obtain a case for teaching purposes except in the spring

Professor YLPPÖ (Helsinki) To return to the general feeding problem it is often very difficult to say which kind of feeding is the best In Helsinki we have two wards for premature children one in the Children s Castle and one in the Children s Clinic I am the chief of both We feed breast milk from wet nurses and from the second week on add 10 per cent cow s milk to every meal in both institutions Yet the results are quite different In the Children s Clinic we had a mortality rate nearly twice that in the Children s Castle *During the past year the results have suddenly changed* In the Children s Castle infections became common and our results there were worse than in the Children s Clinic This shows how much the fate and development of prematures depend on the incidence of infection in different hospitals or different countries

Professor WALLGREN (Stockholm) I wonder if what is fed is as important as the skill with which the babies are treated Listening to these different opinions one admires the great tolerance of premature babies

Professor PLUM (Copenhagen) Dr Magnusson what proportion of the gain in weight could be accounted for by the nitrogen retention *in your* experiments?

Dr MAGNUSSON (Stockholm) I cannot answer Professor Plum s question

In collaboration with Dr Wretlind I have carried out experiments on a few premature infants comparing unsplit and hydrolyzed human plasma as a supplement to breast milk Plasma hydrolysate produced a distinctly greater weight gain than normal plasma The latter had a salt concentration of 12 per cent and some infants became edematous

Professor WALLGREN (Stockholm) We used dry plasma added to breast milk for our premature babies for some time and we had the same experience They gained in weight and we were happy about that but in some cases edema developed and so the plasma feedings were stopped

Dr BRAESTRUP (Hellerup) I have used native human serum obtained from freshly coagulated blood of the father

Dr WRETLIND (Stockholm) As far as I know native plasma is very slowly hydrolyzed by proteolytic enzymes

Dr BRAESTRUP (Hellerup) I had heard that these proteins could be absorbed without being digested but after our experiments I did not feel his could be confirmed

Professor RÄIHÄ (Helsinki) I should like to ask Dr Magnusson if the amount of sugar was the same in the Aminosol and casein feedings

Dr MAGNUSSON (Stockholm) The only difference between the periods was that in one the casein was split and in the other unsplit The amount of carbohydrate given was exactly the same

Professor RÄIHÄ (Helsinki) We obtained results similar to yours by feeding a constant protein diet but raising the carbohydrate Higher nitrogen retention resulted

Dr MAGNUSSON (Stockholm) Dr Haas of Freudenberg s Clinic Basel demonstrated in his investigations that the weight increases brought about by an amino acid preparation cannot be explained solely as the result of the increase in calories

Professor WALLGREN (Stockholm) I believe this was a very enlightening discussion We pediatricians ought to know what to give our premature infants to increase their protein retention but the question is not only a scientific one It is also a question of what is simplest and most economical Aminosol is not very economical If it is possible and it looks as if it would be to feed premature babies with cow s milk and get the same results as with human milk supplemented with protein unsplit or hydrolyzed I believe we shall have to change our present conceptions I have no answer to this question now but I think it is very important

Dr MAGNUSSON (Stockholm) The daily cost for Aminosol for a pre

mature infant amounts to less than 10 cents The difference from the economic point of view between unsplit casein and casein hydrolysate can therefore be considered to be of no account especially when it is viewed in the light of the high costs (about 8 dollars a day) incurred for other needs in connection with premature infants

Professor LEVINE (New York) Should we aim for the greatest possible weight gain daily in feeding premature infants?

Professor WALLGREN *(Stockholm)* *Some 20 or 30 years ago* there were articles in the German medical literature on this question and the authors were of the opinion that one should not try to get as much increase as possible in weight but that there should be a moderately high increase We know for example that the tendency to rickets is much higher when the weight increases very rapidly as was already mentioned

Dr BRAESTRUP (Hellerup) It is an important economic question in Denmark to get premature infants to increase in weight rapidly It costs something like 30 to 35 crowns daily to have them in the hospital and we cannot send them out before they weigh at least 2500 gm

Professor WALLGREN (Stockholm) This is a very interesting point Should prematures grow as fast in extrauterine life as they do in the uterus at the same age?

Professor BARNETT (New York) There are some data relevant to this point If one studies a normally growing baby determines the amount of electrolytes and water retained and tries to relate this to what *should* be retained from our estimates of normal body composition the differences between what is and what should be are almost completely inexplicable

Professor LEVINE (New York) Indirect evidence as to the amount of gained weight deposited as protein which Professor Plum asked about might be obtained from our experiments in which the amount of protein retained was measured when human or cow s milk was given at different levels of intake With the higher protein intake whether in the form of human or cow s milk higher amounts of nitrogen were retained as determined by balance studies *One finds that the ratio of water retained to the total* body weight gain is 0 65 whether the protein intake is low or high indicating that 65 per cent is the water content of the infant

On the other hand as pointed out in Uppsala nitrogen balance studies are not adequate for this type of investigation One would have to study amino acid nitrogen balances because more nitrogen may be excreted in the form of a high content of amino acids rather than urea when cow s milk is given than when human milk is given Such studies are just beginning

There has been some work done that indicates that in dogs nitrogen bal

ances improve when fibrin hydrolysates are used as against casein hydrolysates To my knowledge nobody has made fibrin hydrolysate studies of growth in premature infants Could you make a comment on that Dr Magnusson?

Dr MAGNUSSON (Stockholm) *As far as I know no investigation using fibrin hydrolysate has been carried out on prematures*

HUMAN AND BOVINE MILK IN THE FEEDING OF PREMATURE INFANTS

Professor LEVINE (New York) Theoretically the nutritional needs of premature infants can be met better by cow s milk mixtures than by human milk for the following reasons

1 Unmodified human milk is a relatively dilute feeding and more concentrated intakes are less likely to precipitate vomiting abdominal distention respiratory distress and diarrhea in an organism already handicapped by a lowered tolerance of the gastrointestinal tract

2 The fat content of human milk is high and many premature infants exhibit difficulties in alimentary absorption of this food constituent

3 The relatively low concentrations of calcium and especially phosphorus in human milk may be inadequate for bony growth in a rapidly growing skeleton with an already deficient fetal storage of these minerals

4 Finally the low protein content of human milk may not meet the needs for deposition of new protoplasm in a rapidly growing organism

These physiologic considerations demanded reassessment of the long standing clinical impression that human milk is the food choice par excellence for premature infants

A clinical study was therefore made by Dr H H Gordon of three groups of 122 premature infants receiving isocaloric feedings of human milk evaporated cow s milk and skimmed cow s milk mixtures The composition of these feedings is shown in Table 42

To provide intakes of 120 calories per kilogram daily 180 cc of human milk are required in contrast to 150 cc or less for the cow s milk mixtures The respective feedings furnish increasing proportions of protein from 2 to 6 gm and decreasing proportions of fat from 6 7 to 2 2 gm The carbohydrate content as might be expected is highest in the skimmed cow s milk mixture

The infants whose birth weights were between 1000 and 2000 gm were rotated on the feeding schedules on the basis of their birth weight color and sex Each feeding schedule provided 120 calories per kilogram of body weight per 24 hours after the first week of life a level which was maintained by adjustments in relation to weight every three or four days Observations

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on body weight gain extended over a three week period from 7 to 28 days of age The results are shown in Figure 199

For 16 infants fed human milk the average gain in weight was 12 5 gm per kilogram daily as compared with 14 1 gm for 39 infants fed the evaporated milk mixture and 15 7 gm for a slightly larger group of infants fed a half-skimmed cow s milk mixture These differences were statistically significant When the groups were subdivided into smaller and larger infants (those weighing 1022 to 1621 gm at birth and those weighing 1621 to 1996 gm at birth) the differences in the smaller infants were even more striking 11 7 gm weight gain per kilogram daily for those fed human milk 14 9 gm for the evaporated milk group and 17 3 gm for the infants given the half skimmed milk mixture In the larger infants differences among the three mixtures were smaller and not statistically significant

TABLE 42

Feeding Mixtures for Premature Infants

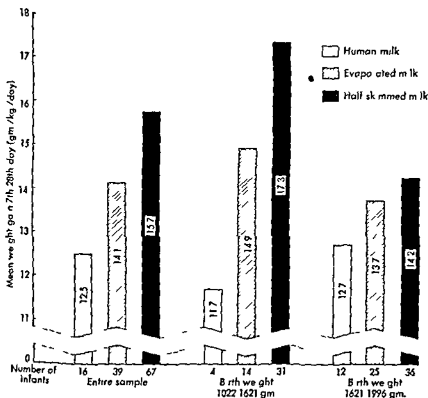
120 cal /kg –55 cal /lb

	Per Kg	Per Lb	Gm /Kg		
			P	F	C
Human Milk	180 cc	$2\frac{1}{2}$ oz	2 2	6 7	13
Evaporated Milk CHO Water q s ad	70 cc 6 gm 150 cc	1 oz 3 gm $2\frac{1}{4}$ oz	4 8	5 5	13
½ Skim Milk Powder CHO Water q s ad	18 gm 11 150 cc	1 T 5 gm $2\frac{1}{4}$ oz	6 0	2 2	19

Both laboratory and clinical studies therefore establish that in hospital practice under controlled conditions of cleanliness temperature and good nursing care cow s milk properly modified is superior to human milk in promoting weight gains in premature infants partially skimmed milk formulas being best suited for the smaller infants

It should be stressed that this conclusion does not necessarily apply to large prematurely born and full term infants who are strong enough to suckle the breast directly Breast milk remains the food of choice for this group be

cause psychologic bacteriologic and economic factors far outweigh the cited advantages of cow s milk But it is to be remembered that milk obtained directly from the breast is wholly different from human milk collected by manual expression or electric pump pooled heated frozen warmed before feedings and fed by bottle Human milk so processed is as artificial a feeding as cow s milk similarly processed



F g 199 Premature infants fed 120 calories per kilogram of body weight da ly

The value of skimmed cow s milk mixtures providing 120 calories 150 ml of fluid 6 gm of protein 2 gm of fat and 19 gm of carbohydrate per kilogram of body weight per day is attested by the fact that from 1940 to 1950 73 premature infants who weighed 1000 gm (2 2 lb) or less at or soon after birth have survived and thrived on this feeding The survival rate for this total group of miniature infants was 28 per cent (73 of 263 infants) for the ten years and 38 per cent (51 of 135 infants) for the last four years

Their weight gains and other statistics are shown in Table 43

If one assumes that the ideal weight gain of infants delivered prematurely should correspond in their early postnatal life to their weight gains had they remained in utero the reported figures gain added significance

Both Scammon and Huggett report that a fetal weight of 1000 gm the birth weight of the infants under discussion is reached by the seventh lunar month of pregnancy and that the daily weight gain during the eighth lunar month of pregnancy averages 22 5 gm (19 6 to 25) and per kilogram of

TABLE 43

Weight Gains in Premature Infants Fed Partially Skimmed Cow s Milk Mixtures

Year	Infants			Ave Gain in Grams	
	Total	Survivors		Per	
		No	Per Cent	Day	Kg /Day
1940–1945	96	15	16	24 4	13 6
1946	31	7	23	24 0	13 9
1947	28	10	36	26 6	15 3
1948	33	10	33	24 7	14 3
1949	46	18	40	23 9	14 1
1950	28	13	46	24 8*	13 4*
TOTAL	263	73	28	24 6	14 1

* One infant not included—still in residence

Range—Days of		Range—Wt —Grams	
Full Feeds†	6–42	Birth	662 1280
Discharge	51 131	Lowest	555–1000
Period of Observ	41–107	Discharge	2140–3075

† Day of age full feedings (120 cal /kg) reached

body weight 15 0 gm (14 3 to 15 5) These figures are virtually identical with those actually observed in this study and thereby give further support to *the use of skimmed cow s milk mixtures as a valuable diet for small premature infants*

The graphic record of one of these infants is illustrated in Figure 200

This female Negro infant was born in April 1941 after a gestation period of six months weighing 662 gm (1 lb 7 oz) She reached a minimum weight of 588 gm (less than 1 lb 5 oz) on the fourth day of postnatal life Thereafter she gained weight at an average daily rate of 22 gm reaching 2660 gm (5 lb 14 oz) upon discharge on the ninetieth day of life

At six days of age she was changed from feedings of human milk to the previously mentioned skimmed cow's milk mixture. Since 1941 this mixture has become the routine initial feeding for all small premature infants.

The daily caloric intake at no time exceeded 120 per kilogram and this level was reached only by the ninth day to obviate potential digestive and respiratory difficulties. The daily fluid intake exceeded 140 cc per kilogram only on the seventh and eighth days and after 80 days of age and ranged in

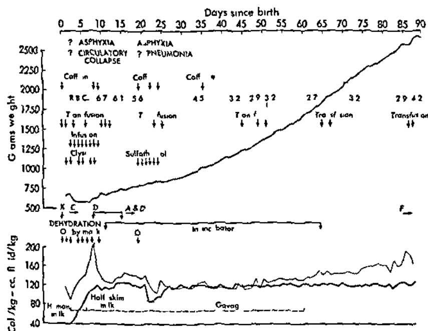


Fig 200 Care and feeding of a premature infant (Gordon H H and Levine S Z *J Pediat* 24 474 1944)

the interim between 120 and 140 cc per kilogram. The infant was fed solely by gavage for the first 61 days of life without evidence of irritation.

A follow up study of this child made in September 1950 showed her to be an essentially well child with normal physical and mental development.

The evidence presented in the introductory talk at this session suggests the following choice of feedings in hospital practice for premature infants of different birth weights.

1 *For premature infants weighing less than 1750 gm* half skimmed cow's milk mixtures high in protein and low in fat

2 For premature infants weighing more than 1750 gm but unable to suckle the breast directly evaporated or boiled whole cow s milk mixtures

3 For premature infants who are strong enough to nurse breast feeding

The data do not prove that the regimens as outlined are necessarily ideal

It should be emphasized that although human milk is not the food of choice for small premature infants in the hospital every effort should be made to maintain the mother s milk supply so that the infant can be put to the breast when he is discharged home Breast feeding is one of the best measures for allaying the anxiety which is prone to develop in the mother of an infant who has required so much special care at the time of birth and while in the hospital In addition it is cheaper cleaner and less time-consuming Finally it is the food provided by nature and may possess certain as yet unidentified biological advantages

Studies now in progress throughout Europe and the United States may show that other modifications such as human milk plus casein or casein hydrolysates may be as satisfactory or even more so as the regimens recommended in this report

Professor WALLGREN (Stockholm) If medical students and student nurses are taught that breast milk is the best food for a young baby and yet premature babies are given cow s milk mixtures will not this seem contradictory? It may lead to continued bottle feeding of all babies

How many of these premature infants were white and how many Negro?

Professor LEVINE (New York) About 85 per cent were white

I think what Professor Wallgren has said is exceedingly important and in Europe where human milk is readily available nothing should be substituted for it However Dr Magnusson s work and that of others indicate that in the small premature infant it may be desirable to modify human milk rather than to give it in its natural state I should guess that one can obtain the same results with modified human milk as with modified cow s milk If we had all our mothers in the United States nursing their babies as you do here the first study that I would undertake would be to make a comparison in premature infants of low birth weight between modified human milk and modified cow s milk

Professor WALLGREN (Stockholm) We do not believe that human milk is an adequate food for premature infants Would not the most adequate natural milk for premature infants be colostrum? I use human milk modified by adding protein and glucose

Professor YLPPÖ (Helsinki) If a premature infant is being fed human milk for which cow s milk is substituted the next day the child s alkaline urine will be acid and the acid stools alkaline We can say the stools are un

important but the urine is a product of metabolism So we have produced a metaboli m with acid by products instead of alkaline ones This is never a natural condition for a child as we know from patients with diabetes Of course we can get larger increases in weight with cow s milk but as has already been pointed out weight is not necessarily our best criterion A fat person is not always a healthy one And in Finland we have concluded that with cow s milk premature infants contract rickets more easily and have convulsions more often There remain many unanswered questions which keep us from saying that increase in weight is the best guide to what we should feed

Dr ROTHE MEYER (Copenhagen) For several years our investiga tions have had the aim of finding an adequate protein supplement for human milk We started with half-skimmed acidified milk and were able to confirm the results of Levine and Gordon finding a greater weight gain with this mixture than with human milk Amino acid supplements to human milk did not yield good results but the Danish amino acid preparations were not very good In the last year and a half we have used unsplit casein as a supplement to human milk and we have had very satisfactory preliminary results In all these experiments our criterion of a good mixture has been gain in weight

Figure 201 shows a comparison between premature infants fed human milk half skimmed citric acid cow s milk or a mixture of human and cow s milk—*allaitement mixte*

The total plasma protein level decreased from the age of 10 to 50 days no matter which feeding was used The decrease was largely due to a decrease in the globulins the albumin fraction staying almost constant

However the total plasma protein level was even lower in this period in prematures fed human milk than in prematures fed cow s milk mixtures This difference was due to a slightly lowered albumin in the human milk fed infants with no significant difference in the globulins

We also found that in human milk fed prematures during the same period the blood urea decreased up to about four weeks of age and after that was constant This decrease was not observed in the group fed citric acid milk or *allaitement mixte* so that the level of blood urea in prematures fed human milk was significantly lower than the level of blood urea in prematures fed cow s milk mixtures This might indicate that cow s milk puts a greater strain on the kidneys but as all the levels are within the normal range one should be cautious in interpreting this difference as abnormal

Dr BRAESTRUP (Hellerup) Professor Levine what is done with the milk of the mother if she has any?

Professor LEVINE (New York) In every instance we pump the breast

of the mother while the premature infant is in hospital hoping that the baby may be discharged to breast feeding May I repeat my conviction that direct breast feeding following discharge has advantages that outweigh any of the chemical advantages that cow s milk might theoretically possess

Professor RÄIHÄ (Helsinki) Our mortality from infection has been so high that we have no material to compare with Professor Levine s

Can the high nonprotein nitrogen be explained as being due to greater formation of carbohydrate when using a high protein diet If so this would

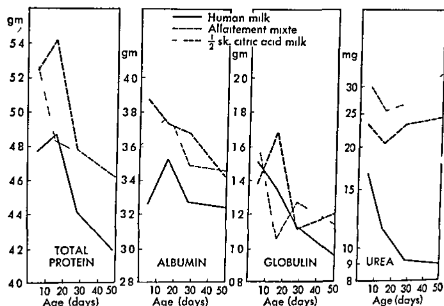


Fig 201 Means of total protein albumin globulin and blood urea in premature infants from seventh to fiftieth day of life (Rothe Meyer A *Acta paediat* 38 556 1949)

agree with our results that a high-carbohydrate diet results in increased nitrogen retention and increased water intake Is some part of this rapid gain in weight just deposition of water? Is there any information concerning the water content in breast fed and cow s milk–fed children?

Professor LEVINE (New York) We have observed the high urea levels too and it may mean that the kidneys of a premature infant are under an increased load because of this We shall be able to evaluate better the utilization of bovine and human milk proteins when studies are made of amino acid excretion in the urine of premature infants fed cow s and human milk

The ratio between the weight of water deposited in the body and the total

body weight gain of infants fed human milk and infants fed cow s milk are exactly the same 0 65 This is roughly the water content of protoplasm

Professor STEARNS (Iowa City) I object very strenuously to anyone saying that human milk is more economical than cow s milk Because if the mother is to maintain her own body she has to take the extra cow s milk and vitamin D herself

The infant normally stores calcium during the last two months of pregnancy in preparation for the drop in calcium content that always follows birth This drop is greater with human milk because of the lower calcium content The prematurely born infant misses out completely on that period of heavy storage As soon as we can make that up to the body he becomes a normal infant again and can get along well with human milk

Professor WALLGREN (Stockholm) Should the basic feeding be cow s milk or human milk?

Professor STEARNS (Iowa City) We use human milk and add dried skim milk to it

Professor WALLGREN (Stockholm) Professor Levine is of the opinion too that if they had human milk available in his clinic it would be the basic feeding supplemented with what is necessary for the premature infant I suppose that is something that all of us agree upon human milk is not an adequate feeding for premature babies but cow s milk is not an adequate feeding either

Dr von SYDOW (Sundsvall) I should just like to refer to two diagrams one of which was Figure 59 (p 106) of the Panel on Calcium Phosphorus and Vitamin D That diagram showed the serum values for phosphatase inorganic phosphorus and calcium in four groups of premature infants given different feedings Vitamin D alone had a pronounced effect on the serum calcium values but only a very slight influence on the phosphatase and phosphorus values The addition of cow s milk on the other hand did not alter the serum calcium at all but it increased the serum phosphorus values greatly and lowered the phosphatase values The addition of both cow s milk and vitamin D finally improved all three values and made them practically the same as in the normal series

Figure 202 shows the correlation between the serum values and the roentgen findings in the wrist The black columns as before represent the normal group In the cases of phosphatase and inorganic phosphorus the results are recorded according to whether breast milk or cow s milk had been given and independently of whether or not vitamin D had been given since this appeared to have no great influence on the phosphatase and phosphorus values For serum calcium the results are recorded according to whether or not vita

min D had been given no consideration being taken of whether cow s milk had been given So the transversely hatched columns in the left and center parts of the diagram represent prematures given cow s milk and the longitudinally hatched ones in the last part of the diagram those given vitamin D The stippled columns with or without hatching mean those cases showing roentgenological signs of rickets and the unshadowed ones those showing normal wrists

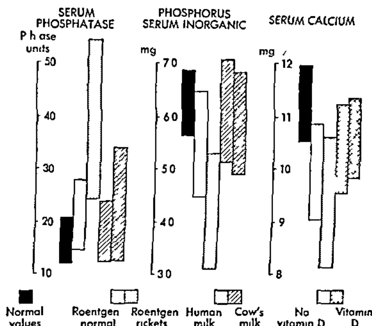


Fig 202 Serum phosphatase phosphorus and calcium values in normal and roentgenologically rachitic premature infants (von Sydow G *Acta paediat* 33 [suppl 2] 108 1946)

In those infants given human milk the phosphatase values were much higher and the phosphorus values much lower particularly if roentgenological rickets was present In those given no vitamin D the serum calcium was lower if rickets was present There was no significant difference between roentgenologically rachitic and normal infants with respect to the phosphatase and phosphorus values if cow s milk had been given, or with respect to the calcium values if vitamin D had been given

It seems to me that these findings may be interpreted in this way the absorption of calcium from the gut is much influenced by vitamin D and the absorption of phosphorus by the amount of phosphorus available in the food In some way this is reproduced in the formation of bone so that when the absorp-

tion has been poor and the serum values are abnormal then the bone is prone to become rachitic

This seems to indicate that both vitamin D and also cow s milk as the best source of minerals have to be administered to premature infants in order to prevent rickets This is increasingly true the more premature the infant is for the difference beween the feeding groups is much more pronounced in the lowest birth weight group It may also apply to a certain extent to full term infants who for some reason have not stored sufficient amounts of minerals in their body before birth

From this I have not however drawn the conclusion that premature in fants should be given cow s milk instead of breast milk but only that they should be given a certain amount of cow s milk as a supplement to the breast feeding In my practice infants with a birth weight of 2000 gm or less are given a supplement of about 100 gm of cow s milk from the second week of life until they are able to take their needs themselves from the mother s breast At that time the infant usually has replenished its mineral stores to a certain extent and the breast milk consumed seems to prevent any considerable further decrease

Professor WALLGREN (Stockholm) Dr von Sydow implies that different conceptions concerning cow s milk and human milk as a basic food for premature infants might be reconciled in this way use human milk supplemented by cow s milk

Professor BARNETT (New York) I would like to mention several points that take us back to the earlier discussion the first of which concerns the relation of protein intake to blood urea nitrogen which Dr Rothe Meyer discussed

We have some observations which indicate that the higher blood urea nitrogen found in infants on cow s milk is purely a function of the amount of protein taken in rather than any difference in the way this protein is metabolized We were amazed to find values of blood urea nitrogen in normal infants as high as 60 or 70 mg per 100 ml when they were given diets containing 9 gm of protein per kilogram Whether this high level of blood urea nitrogen imposes a load on the kidney which is harmful cannot I believe be answered at the present time

Apart from the pedagogical effects which Professor Wallgren mentioned it seems to me that the advantage of using human milk as the basic food for premature infants has not yet been proved We all tend to accept it on a basis which has not been experimentally validated except in the psychological sphere If the higher fat content is a disadvantage which means that the milk has to be processed to lower the fat then even the possible immunolog

ical advantages will I think be in large part lost because the milk will be heated

Professor SALOMONSEN (Oslo) It is misleading to consider this problem solely from the nutritional point of view There is another factor which I think is very important namely the content of antibodies in human milk Infections are the main cause of mortality in premature infants and in that respect cow s milk will never be as good as human milk Professor Levine do you think that boiling mother s milk will destroy its antibody activity?

Professor LEVINE (New York) *Professor Vahlquist in Uppsala indi* cated that diphtheria and tetanus antitoxin at least are not transmitted through human milk but by the placenta There may be other antibodies or *factors, transmitted through human milk but I do not think even that has* been fully established In the last ten years when we have used only cow s milk we have never had any infections in the premature nursery

FAT ABSORPTION STUDIES IN PREMATURE INFANTS

Dr SÖDERHJELM (Uppsala) Human milk has usually been con sidered the food of choice for premature infants Recently however mix tures of cow s milk which are poor in fat but rich in protein have been recom mended as being especially suitable for this purpose This change in part at least has been based on the common belief that premature infants do not absorb fat well hence breast milk relatively rich in fat is less desirable for prematures than for full term babies Actually however there is no com plete agreement with regard to the superior quality of one or the other type of milk The purpose of this paper is to report fat balance studies in prema ture infants who were fed either human milk or cow s milk

Historical A number of workers have reported greater absorption of human milk fat than of cow s milk fat by healthy full term infants Factors which have been shown by Holt and co workers to influence the absorption of fat in the healthy full term infant are age of the child composition of the milk fat and the mineral content of the milk In their study fat absorption was found to increase with increasing age and with an increasing proportion of unsaturated fatty acids in the milk fat There appeared to be a decreased absorption as the mineral content of the milk increased The size of the fat globules was of no significance ordinary milk being absorbed to the same degree as homogenized milk They reported a tolerance of 10 gm fat per kilogram daily by infants 5 to 10 months of age Lindberg and Reinhold stated that when the fat content of breast milk was increased to about 7 per

cent vomiting and gastrointestinal disturbances resulted and Van Espen reported increased vomiting with increased fat content of the breast milk

Rubner and Langstein in 1915 were the first to study the absorption of fat in premature babies fed breast milk The fat absorption was a little low in one of their patients and very poor in the other (the lowest fat absorption so far found in a healthy premature infant) Rubner and Langstein seem to have determined the fat absorption indirectly from the metabolism of nitrogen and carbon weight gain and oxygen consumption It is therefore difficult to evaluate their results Both infants got large volumes of milk with a high fat content

Gordon Levine and co workers found rather poor fat absorption in their seven short but very carefully performed balance studies In children weighing more than 2000 gm the fat absorption was satisfactory

Flori in Italy found excellent fat absorption in eight premature infants although they were fed large volumes of milk and much fat

There is considerable discussion in the literature on the changes produced when human milk is heated and also on the relative value of raw versus boiled or heated breast milk In some instances the fat absorption was studied Catel found a little lower but still above 80 per cent absorption when the milk was boiled Blume Westerberg did not find that boiling influenced the fat absorption One of her infants absorbed only 75 per cent of the fat but received 10 gm fat per kilogram of body weight per day

These earlier investigations thus show good fat absorption with the exception of the infants studied by Gordon and Levine and a few other experiments where the infants received large volumes of milk

Material and Methods The present report describes some simple fat balance studies carried out in Uppsala and in Galveston Texas Although minor details of technique differed slightly in the two places the main features were similar

In Uppsala the premature infants were given breast milk either the mother s in which case it was not heated or pooled breast milk which had been pasteurized at 72° C for five minutes or heated for three minutes in boiling water (97 C)

In this series 31 separate fat balances of four to five days duration were carried out on eight premature infants weighing 960 to 1960 gm Since the passage of food through premature infants is rather rapid usually requiring less than 24 hours it was felt that the collection of the feces over a definite time during which the fat intake was known would give data sufficiently accurate for this study Long balance periods required for older children and adults do not appear necessary for prematures The fat content of the milk

was determined daily using Gerber s method The total fat of the wet feces was extracted with warm acid alcohol and ethyl ether

In the work begun at the University of Texas Medical Branch and later continued in Uppsala the premature infants were given either human milk or cow s milk The effects on the fat absorption of freezing or heating the milk were examined

In this series 67 separate balance studies were conducted on 24 premature infants weighing 1080 to 2220 gm The periods were of four to seven days duration The infants were kept in incubators and fed 140 to 190 ml milk per kilogram per day The effects of the following treatment of the milks were studied (1) human milk untreated frozen * pasteurized for 20 minutes at 62° C heated on a boiling water bath for 20 minutes at 97° C or heated in an open pan for 3 minutes at 100° C (2) whole cow s milk pasteurized for 20 minutes at 62° C (homogenized in 2 instances) or boiled in an open pan at 100° C for various lengths of time Carmine and charcoal were used to mark the beginning and end of each period and it was found that the rate of passage of these markers in the premature infant was the same The fat content of the milk and that of the wet feces were determined daily by using the Mojonnier method

Discussion of the Methods Used Careful investigations by Gordon Levine and co workers showed a maximum loss of 5 per cent of the milk given to premature babies This was due to regurgitated milk and milk left in bottles tubes and the mouths of the babies In our experiments smaller volumes of milk were used and regurgitation though rare was estimated and deducted from the milk given The losses of milk in the procedures carried out in these investigations amounted to a maximum of 2 5 per cent This small percentage would influence the figures of fat absorption only slightly

The most difficult problem is the complete collection of the stools We have not restrained the infants Cellulose or paper has too much lipoid material for use in collection of the feces Holt and co workers extracted the diaper with the stools and deducted 0 5 gm of fat for each diaper which is a high figure compared to the fat content of the feces Some of the infants in my experiments were placed on plastic sheets but the results were not different from those with common cotton diapers Infants with diarrhea were not included in these studies

To find out how much was lost in the diapers I have spread out feces of known fat content from breast fed infants on diapers and studied the losses on repeated collection In this procedure not more than 4 to 5 per cent of

* Pooled milk autoclaved at 114 C then frozen 2–6 months or pooled milk frozen 1–2 months heated 5 min at 72 C

the fat of the feces was lost although the feces were mixed with water and thinner than normal stools of breast fed infants

The methods used here for the determination of fecal fat give the total fat and not just the saponifiable fat The difference between the total fat and saponifiable fat was determined in several cases according to Kamer s method The difference was always found to be more than 5 per cent of the fat content Thus the possible losses in the diapers are fully compensated

Results The absorption of fat from human milk in the first series was excellent Heating the milk to 72° C for five minutes or to 97 C for three minutes did not influence the degree of absorption In these infants who received 3 3 to 6 4 gm fat per kilogram per day there was no significant difference in the degree of absorption of the fat The mean absorption for the group was 92 per cent which is as good as for full term infants The weight gains during the individual periods were not directly related to the amount of fat absorbed

The results of the fat balance studies in the series using breast milk which had undergone various heat treatments were almost the same as in the first series with one exception This was true even though the heat treatment was more severe as for example 20 minutes at 62° C or 97° C Freezing and storage of breast milk had no influence on fat absorption In only one instance was the per cent of absorption low yet when the same infant weighed 120 gm more with a greater fat intake the per cent fat absorption was increased Frozen breast milk which consisted of pooled milk which had been autoclaved at 114° C before freezing and subjected to an additional heat treatment of pasteurization before use appeared to be just as satisfactory in regard to fat absorption by premature infants as the fresh milk heated for five minutes at 72° C before use

The premature infants who were given cow s milk pasteurized or heated for various intervals with intakes of fat of 3 8 to 7 8 gm per kilogram per day retained fat to a considerably less extent than the infants fed breast milk The retention of fat in 22 experimental periods observed in six different premature infants was about 70 per cent on the average and the type of heat treatment did not alter the retention

Discussion Absorption of fat from both breast milk and cow s milk by premature infants seems remarkably good when the amounts given are in the range of 3 to 7 gm per kilogram per day Even small premature infants seem to retain fat well Nine infants weighing less than 1500 gm absorbed 91 per cent of the fat from breast milk The degree of absorption was not altered by the treatment given the milk (pasteurization freezing or heating) With cow s milk fat absorption was about 66 per cent when the fat was given

at a level of about 4 gm per kilogram per day The degree of absorption of fat from cow s milk by the small premature infants is not much different from that of the larger premature infants Both smaller and larger premature infants however retain fat from breast milk with greater facility than from cow s milk

Gain in weight usually is used as a criterion of effectiveness for feeding the premature infant Levine and Gordon noted that premature infants showed greater increase in weight when fed isocalorically on a cow s milk mixture which was high in protein and low in fat (2 0 gm per kilogram per day) than when given milk of higher fat content (4 4 gm per kilogram per day) Difficulty in fat absorption by prematures has been emphasized by these workers as one of the important problems to be met in proper feeding Ford however reported satisfactory weight gain in prematures maintained on breast milk Öberg and Ågren found no difference in the weight gain of premature

TABLE 44

Fat Absorption in Premature Infants Fed Human Milk

	No Periods	80 Per Cent	80–90 Per Cent	90 Per Cent	Median Per Cent
Found	76	5	31	40	97
Corrected	76	9	28	39	91

infants fed isocalorically on breast milk or formulas richer in carbohydrate and protein If it is true that premature infants actually do increase in weight more rapidly when fed on diets low in fat it cannot be stated that this is attributable to the inability of the premature infant to absorb fat satisfactorily The data herein reported show that the premature infant retains breast milk fat practically as efficiently as has been reported for the full term infant

Thus the absorption of breast milk fat was studied in 26 premature infants in 76 periods of 3 to 6 days duration The results are summarized in Table 44 The corrected values are the results obtained if 5 per cent of the fat in the milk and 5 per cent of the fat in the feces are assumed lost

REFERENCE

Soderhjelm L *Acta paediat* 41 207 1952

Professor GYLLENSWÄRD (Stockholm) I have some material contributing to the question of feeding and resistance to infections gathered from

two groups of infants cared for in a children's home One group consisted of children breast fed by the mother and the other group was bottle fed Both groups came from very low income groups All were full term babies and I found that there was a marked difference between the two groups The breast fed children gained in weight much better than did the bottle fed children although both groups gained less than the subjects described in a paper by Dr von Sydow The breast fed and bottle fed infants contracted infections similar to those which Professor Ylppo mentioned Also the rise in temperature when the child was ill was about the same in both groups

When evaluating results one must be very careful to consider the differences between the sexes A female has much better resistance and ability to survive than a male In Sweden I have found that the stillbirth rate is 20 per cent higher for males than for females and the neonatal mortality is bout 30 per cent higher in males than in females Thus a girl who weighs 2400 gm is not as premature as a male of 2500 gm Perhaps too the better resistance of Negro children can also be explained by a higher degree of maturity for the same birth weight than white children have

Professor LEVINE (New York) Professor Gyllenswärd is correct The degree of maturity is not the same for a given birth weight unless sex and race are considered The female infant and the Negro infant are both more mature with relation to weight That was taken into consideration in our observations for all the infants were paired with relation to sex and color as well as to birth weight

We do not know enough yet of the differences in resistance of premature babies to infection when fed on human or cow's milk Differences may be dependent on different bacterial flora in the intestines on the transmission of antibodies some of them as yet unknown or on other factors that we know nothing about now I think the only way that one can establish differences is by proving them

With regard to the discussion this morning we all agree that body weight gain per se is not the best index of the best type of feeding I should think that the survival rate and mental physical and emotional development over a long term period would be our best criteria The crucial observations will be long term studies on modified human and modified cow's milk with careful measurements and evaluation of the development of premature infants on the different types of feeding

Dr von SYDOW (Sundsvall) Some years ago Dr Magnusson and I made a comparison similar to that made by Professor Gyllenswärd between breast fed and bottle fed infants at an infants home in Goteborg Our breast fed infants however were not nursed by their mothers but were given pooled

breast milk We fed every second child coming to the home on pooled breast milk and the others cow s milk mixture We compared the incidence of infection and found that there was only a slight, and not statistically significant superiority for the infants who got pooled breast milk

Professor YLPPÖ (Helsinki) We gave our premature infants the smallest amount of milk which caused a satisfactory gain in weight and did not calculate caloric intake We also tried giving these infants skimmed breast milk but loose stools and a decrease in weight resulted We put more and more sugar in the skimmed milk but could not get any increase in weight However when we gave plain breast milk again the stools became normal the following day Therefore it appears necessary to have fat in breast milk and if there is only 1 to 2 per cent fat the babies have loose stools

Dr SÖDERHJELM (Uppsala) At the maternity hospital which I visited in America it was the custom to send the mothers home on the third day after delivery The mothers had very little breast milk at that time very few wanted to suckle their babies and those that did did not want to donate any breast milk Only 5 per cent of the babies left the hospital wholly breast fed

Dr Stevenson of Pittsburgh told me about some experiments with cow s milk–fed babies in the southeastern part of the United States where it was found that the poorer the hygiene the better the results were with breast milk In some parts of Texas the hygiene is not as good as it should be and there is a very high mortality from infant diarrhea in babies who are bottle fed

Dr BRAESTRUP (Hellerup) Has gain in weight any connection with resistance to infections?

How much water would premature babies of around 1500 gm take if they were allowed to take what they wanted and it was offered by a skilled nurse? It is my experience in such a case that they will generally take more than 150 ml per kilogram of body weight

Professor GYLLENSWÄRD (Stockholm) There is a connection between weight gain and resistance to infection although I do not think it is quite what you had in mind If an infant contracts an infection he does not gain as much weight as when he is free of infection In Sweden years ago it was shown that in a large ward with 15 or 20 infants if one child had an infection most of the other children stopped gaining weight at the same time Furthermore all of us know how careful we must be not to expose dystrophic infants to infection because they fall ill so much more readily than children with normal weight If such infants do contract an infection they are much more severely ill than normally developed infants and the death rate is very high

When one has recovered from an infection he will still be more susceptible to infection for some weeks

Professor LEVINE (New York) My observations on fat absorption were primarily made in relation to cow s milk fat but in the few observations that Dr Gordon made with human fat there still seemed to be some difficulty in absorption An explanation for the discrepancies between our work and that of Dr Soderhjelm seems difficult to find I think we used the same methods as he did but we collected our stools in cans and receptacles instead of diapers and we analyzed dry stools

Dr MAGNUSSON (Stockholm) In a study of airborne infections in children s wards we observed that if we placed a dangerous carrier in an open ward we were often able about one day later to obtain the bacteria which this patient carried in cultures of the nose or throat from many of the other patients on the ward A short time after these children had become infected they ceased to gain weight without necessarily showing any other signs of infection This seems to be a common manifestation in connection with infections in premature and young infants

Professor BARNETT (New York) In the observations of weight gain and frequency of infection in breast fed and bottle fed infants for instance in the home that was described by Professor Gyllensward were bottle fed infants fed by the mothers or by nurses? Also were all the infants in the same nursery? I raise these questions because I think it is extremely difficult to interpret differences in the incidence of infection as due to a single factor when so many are involved As an example I might cite the experience in New Orleans in which an epidemic of diarrhea occurred in the newborn nursery Only breast fed infants were attacked the bottle fed infants being completely free of infection The epidemic was ultimately found by Dr Wegman to be due to the fact that the material used for cleaning the nipples of the mothers had been contaminated

Professor GYLLENSWARD (Stockholm) In my cases both groups were cared for both by mothers and nurses The mothers had to have free hours to go into the city for example and the infants were then cared for by nurses I should emphasize that the incidence of infection was the same in both groups

Dr von SYDOW (Sundsvall) Both breast milk-fed and cow s milk-fed infants were treated in the same nursery by the same nurses and not by the mothers

Dr ROTHE MEYER (Copenhagen) It is usually stated that the neonatal weight loss in prematures is rather more pronounced than in full term infants

I want to ask Professor Levine about his experience with tube feeding of premature infants because I think that we have been able to reduce this neonatal weight loss very markedly by early and consistent tube feeding

Professor LEVINE (*New York*) I think that the neonatal weight loss of premature infants is proportionately smaller than in full term infants We ourselves do not make any effort to reduce this neonatal loss in weight Many of our infants are not given any fluid by mouth for three days except when they show fever or signs of dehydration And the smaller the premature baby is the longer we wait before we feed him by mouth After the initial stage of starvation because of the skilled help that we have we use gavage feeding routinely Some babies are given gavage feeding for as long as two months When they are able to suck spontaneously we give them a bottle with the milk

Professor BESSEY (Chicago) The discrepancy in the various fat studies may lie in the methods of calculation In one case the absorption of fat was expressed in terms of the percentage of fat given In Professor Levine s figures the absorption was expressed in terms of the fat excreted If the excreted fat is not given as percentage of intake the increase from 1 gm to 2 gm looks like a very big one But if you calculate it on a percentage basis the difference looks a great deal smaller It may be possible that these two sets of observations were not as far apart as the figures seemed to indicate

Professor LEVINE (New York) I think that is true and we ought to calculate our figures on the same basis to determine whether there was a dis crepancy in terms of percentage absorption

Dr SÖDERHJELM (Uppsala) In our fat balance studies the amount of fat absorbed is the difference between the fat intake and the output in the feces Some experiments both in animals and humans have demonstrated that there is an intestinal excretion of fat even during fasting This excretion is greater in certain diseases such as sprue or when there is a lack of bile in the intestines or else less of the excreted fat is reabsorbed in these cases

In order to study this phenomenon in premature infants some balance ex periments were performed The children received a formula of skimmed milk powder dextrimaltose and sometimes sucrose The balance experiments were performed in the same manner as described earlier The fat content of the formula was 0 1 to 0 2 gm per kilogram of body weight per day In the feces 0 014 to 0 16 gm fat per kilogram per day was found As these infants on a normal diet absorbed 85 to 95 per cent of the ingested fat most of the fat found in the feces on the fat poor diet must have been endogenous The amounts are small but some of the infants in the previous study were found to excrete less than 0 2 gm fat per kilogram of body weight per day and most of this fat was probably not derived directly from the fat of the food

Professor GYLLENSWÄRD (Stockholm) Professor Wallgren spoke a little of the value or perhaps the danger of propaganda I think the astonishing figures on breast feeding from Texas underline what Professor Wallgren said

It is important in discussing the feeding of babies cared for in hospitals not to forget the very large number of babies who are fed outside the hospitals and whose feeding will be influenced by what is done in the hospital

Professor WALLGREN (Stockholm) In Sweden since we have had pediatricians on the staffs of the obstetric clinics the enthusiasm for breast feeding has increased significantly

Professor LEVINE (New York) We are also attempting to propagandize and have increased the frequency of breast feeding in the United States In many clinics as in ours we have prenatal conferences with the mothers fathers obstetricians and pediatricians We also invite the nurses to these conferences because they play a big role in that it is easier for them to give the baby a bottle in the nursery than to take the baby to the mother and then bring it back I would be the last one to discourage breast feeding in full term babies or in premature babies who are strong enough to suckle

Professor WALLGREN (Stockholm) Nobody really has the impression here that you Americans are trying to advocate bottle feeding of all infants What I meant when I made my few remarks at the beginning was that you may do it unintentionally because when the mothers find that in the clinics premature babies—the most delicate of all human beings—are fed cow s milk mixtures they may say Why should we feed our older infants with breast milk when it involves so much trouble for us? We cannot go out or shop and so forth Perhaps this is the reason why you have such a low frequency of breast feeding in America

Professor LEVINE (New York) May I ask the representatives of the Scandinavian countries whether they can give a figure for the survival rate of premature babies under 1500 gm ?

Professor WALLGREN (Stockholm) The latest figures are from Goteborg in 1948–1950 (800 prematures) The mortality rate was 17 to 18 per cent until a body weight of 2500 gm was reached

SOME CLINICAL AND METABOLIC RESPONSES OF PREMATURE INFANTS TO PITUITARY ADRENOCORTICOTROPIN (ACTH) ADMINISTRATION

Professor LEVINE (New York) I have already discussed the effect of purified pituitary adrenocorticotropin (ACTH) and some of the adrenocortical

steroids on a defect in aromatic amino acid metabolism of premature infants In the course of these studies the opportunity was provided to observe the clinical behavior and to investigate other metabolic phenomena in these subjects during hormonal administration *Some of the observations are I think* sufficiently impressive to bring to your attention My associates in this study were Professor Henry Barnett, Dr Warren Bierman and Miss Helen Mc Namara

The group studied consisted of 26 premature infants weighing from 1112 to 2240 gm at birth Hormone therapy was started when the infants were between 11 and 58 days of age and between 1320 and 2500 gm in weight Each infant received by intramuscular injection one or more of seven hormones as listed in Table 45

TABLE 45
Hormone Administration to Premature Infants

	No of Pts	No of Obs	Daily Dose Range (mg)	Days of Study Range	Total Dose Range (mg)
ACTH	17	24	12 5–100	1–10	12 5 500
Cortisone (E)	6	8	50–100	3 5–10	280–760
DCA	2	2	2–3	5–6	12 15
Testosterone	2	2	5–10	7	35–70
Progesterone	3	3	25 100	7	175–700
A	1	1	100	7	700
L	1	1	100	7	700

Six steroids were used in this study in addition to ACTH because of their chemical structure physiologic properties and availability Compounds E and A are examples of the 11-oxysteroids with protein antianabolic glucone ogenetic and eosinopenic properties DCA testosterone and progesterone contain no oxygen atom in position 11 and possess electrolyte regulating androgenic and protein anabolic properties Compound L is 17 hydroxy progesterone with a hydroxy replacing the keto group in position 3 of the *cyclopentophenanthrene ring Its physiologic actions in man are as yet* unknown As other steroids become available they will be studied

In view of the universal agreement that the action of pituitary adreno corticocotropin is mediated in both animals and man through stimulation of

adrenocortical functions the difference in the responses of premature infants to this hormone and to the adrenocortical steroids is of major interest

EFFECT ON MENTAL AND EMOTIONAL STATE Most observers have remarked on the sense of well being alertness and even of euphoria which often accompanies ACTH therapy in older children and adults The change in behavior of premature infants with this form of treatment was also striking

Hyperactivity manifested by restlessness crying and continuous movements was a constant accompaniment irrespective of dosage This excitable state was first noticeable before meals and often was prominent by the second day of treatment It was consistently present both before and after meals in all of the 17 infants by the sixth day of treatment In one infant the degree of overactivity was so great that his body temperature rose to 39 3 The fact that the fever was due to overactivity alone was established by the prompt return to normal temperature upon application of physical restraints

In the absence of objective measurements perhaps the best index of the degree of hyperactivity was the vigor and duration of crying By the end of the second day of hormone therapy three infants were screaming loudly for a large part of the day and night By the end of the third day ten more joined the chorus At times the premature unit was a vocal bedlam One of the infants cried so long and so loudly that he became hoarse The over activity and crying exhibited during ACTH therapy returned to normal pre treatment behavior patterns within the first four days after cessation of therapy

The change in the reactive state of five infants who were distinctly lethargic before therapy to a state of alertness and tenseness during treatment was especially interesting The fact that this improved state persisted after treat ment may have important clinical implications worthy of further study

These striking changes in general behavior were not accompanied by other overt signs of ACTH overdosage such as edema muscle wasting skin atrophy striae hirsutism or other evidences of Cushing s syndrome Blood pressures were not taken because of technical difficulties

The effect of the adrenocortical steroids on these clinical features was by contrast virtually negligible With cortisone three of six infants showed moderate increases in activity in two crying was slightly more prolonged and more intense A prompt return to the normal state followed cessation of therapy No deviations in pretreatment patterns of behavior were observed with the other steroids

EFFECT ON APPETITE The ravenous appetites manifested by prematu infants were one of the most dramatic features of adrenocorticotropin ther Since the infants were routinely kept on constant diets of 120 calories a

ml of fluid per kilogram of body weight per day throughout their residence in the hospital the hunger which developed with hormone therapy could not be appeased This probably explains in large measure their previously described restlessness overactivity and crying in these periods Often within two days after the onset of treatment and always by the sixth day the infants were emptying their bottles in record time and crying for more food immediately after feedings Sixteen of the 18 infants responded in this fashion in 18 of 24 observations In 4 of the 6 remaining observations in which appetite changes did not occur the dosage of ACTH was minimal and of short duration Increases in the usual caloric and fluid intakes of 50 per cent in one infant and 100 per cent in another infant on the last four days of hormone therapy failed to appease their appetites The latter infant continued to howl lustily for more food even though a marked diarrhea resulted from the excess food Withdrawal of ACTH was required to satisfy his appetite with normal intakes and to halt the diarrhea Cessation of therapy was followed by a gradual return of appetite to pretreatment levels which usually occurred on the fourth to sixth day of the postperiods in all of the infants

Again the relatively negligible effect on appetite of the adrenocortical steroids was in striking contrast In only 2 of the 8 observations with cortisone was there any appetite change with all of the other steroids there was no effect on appetite

EFFECT ON BODY WEIGHT Even more consistent than the effect of pituitary adrenocorticotropin on crying and appetite was its effect on body weight The pattern of body weight changes was identical for all premature infants in all observations and at all levels of daily dosage as seen in Figure 203

The pattern may be described as follows

1 Substantial weight gains in the foreperiods of one to two weeks ranging between 11 and 48 gm per day for individual infants and averaging 27 gm per day for all 17 infants in 19 observations

2 Marked reductions in the rate of weight gain in test periods of 5 to 10 days of hormonal therapy in dosages varying from 12 5 to 50 mg per day Weight changes now ranged between gains of 1 to 7 gm per day in 4 observations and losses of 0 to 18 gm per day in the remaining 15 observations the average weight change was a 5 gm loss per day for all infants in the test

3 Markedly accelerated weight gains in the postperiods of one to two weeks following cessation of ACTH therapy ranging from 34 to 90 gm per day for individual infants and averaging 51 gm for all infants Without a single exception the daily increments of weight gained by each infant in these postperiods in all of the 19 observations always exceeded the foreperiod levels being twice as high in 9 and three times as high in 5 of the 19 observations

If however one adds algebraically the body weight changes which occurred in the test and postperiods and compares these combined figures with the fore period levels one notes that the net result of ACTH administration in terms of total body weight change is insignificant Daily body weight gains for the test and postperiods together ranged between 14 and 32 gm for individual infants and averaged 23 gm for all infants as against corresponding figures of 11 48 and 27 gm for the foreperiods The differences are negligible

A comparison of the body weight changes induced by pituitary adreno corticotropin with those induced by the adrenocortical steroids is rewarding

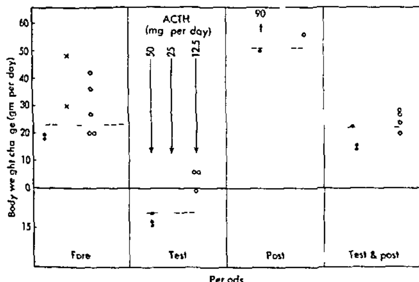


Fig 203 Effect of ACTH on body weight of premature infant

and is shown in Figure 204 It should be pointed out at the outset that the observations thus far completed with the adrenocortical steroids other than cortisone are as yet too few and that additional observations with desoxy corticosterone and testosterone in larger dosage are needed before making definite conclusions

With these limitations in mind the results shown in Figure 204 reveal two different patterns of *body weight change in response to steroid therapy* Fore test and postperiods in all observations were each of at least one week s duration

In all 3 infants receiving progesterone in different daily dosages of 25 50 and 100 mg the pattern of response was consistent and closely paralleled the

body weight response to ACTH therapy namely a marked reduction in th- rate of weight gain in one infant and an actual weight loss in two infant in the test periods an accelerated weight gain in all 3 infants in the postperiods and a total weight change in the combined test and postperiods which ap proximated or was somewhat less than pretreatment levels Gains in weight per day for the 3 infants averaged 29 1 and 35 gm for the three periods and 19 gm for the combined test and postperiods

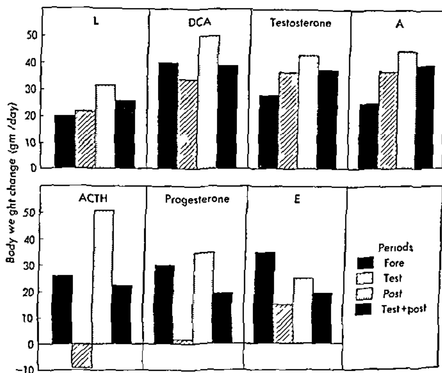


Fig 204 Effect of various steroid hormones and ACTH on body weight of pr mature infants

With cortisone therapy the body weight response of individual infants wa as variable as was the effect of this steroid on the tyrosyluria of premature infants Weight gains in the test periods paralleled or exceeded the levels for the foreperiods in two observations they were moderately reduced in thre observations and they were markedly reduced in the remaining three observa tions These variable results were seemingly unrelated to the level of dosage or to the eosinopenic response or to the effect on tyrosyluria Postperiod weight gains in all eight observations were in general of the same order of magnitude as foreperiods so that the net result for combined test and post

periods was a total weight gain which fell below pretreatment levels The gain in weight for all infants receiving cortisone averaged 35 15 and 25 gm per day for the three periods respectively and 19 gm per day for the combined test and postperiods The effect of this steroid on body weight therefore simulated that produced by ACTH but to a notably lesser degree This *lesser reponse may be related to the greater insolubility of the cortisone prep*aration used or what is more likely to the levels of dosage used in the case of the two hormones

The second type of body weight response differed markedly from the one described above and was qualitatively similar for each of the four other adrenocortical steroids thus far studied desoxycorticosterone testosterone and Compounds A and L It is characterized by a steady and continuous rate of weight gain in both the test and postperiods which was at least equal and in the case of three of the steroids of greater magnitude than the pretreatment levels

The two infants receiving DCA in relatively small dosage gained weight at an average daily rate of 40 gm in the foreperiods 34 gm in the test periods and 50 gm in the postperiods Both infants receiving testosterone in similarly small dosage likewise gained weight steadily throughout the 27 and 32 days of fore test and postperiods the average figures per day being 28 37 and 43 gm for the three periods respectively The single observations with Compounds A *and* L *in a pair of twins are of particular interest* With Reichstein s Compound L the weight gains per day were 20 gm in the fore period 22 gm in the test period and 28 gm in the postperiod With Kendall s Compound A the corresponding figures were 25 gm in the fore period 37 gm in the test period and 40 gm in the postperiod of 5 days

Augmented rates of weight gain with testosterone therapy in premature infants and the absence of androgenic or other side effects have already been reported and put to clinical use If the single observations with Compounds A and L are confirmed by more prolonged studies on a larger number of infants they may have even greater promise in view of their purported absence of androgenic effect in animals Studies with still other protein anabolic steroids are in progress

The weight loss of premature infants in the course of ACTH therapy is explained by *their increased energy expenditure incident to overactivity and* crying which as mentioned before were invariable accompaniments of this form of treatment Further information on the mechanism of this weight loss was obtained by balance studies on each of two infants

Both infants were on constant diets of half skimmed cow s milk mixtures throughout the two weeks of observations Quantitative analyses of their

food urine and feces for nitrogen phosphorus sodium and potassium showed that the positive balances for nitrogen and sodium which were present in the foreperiods became negative in the week of hormone therapy in both infants and that the balances for phosphorus and potassium although still positive in the test periods were markedly reduced The balances for nitrogen phosphorus and potassium in the postperiods returned to pretreatment levels and the postperiod balances for sodium greatly exceeded the foreperiod levels The results of balance studies in these two premature infants therefore confirm the protein antianabolic effect of ACTH previously reported for adults They also suggest that this hormone may play a role in electrolyte regulation in premature infants

EFFECT ON GLYCOSURIA That ACTH also influences the carbohydrate metabolism of premature infants is evident from our studies of urinary sugar Qualitative tests with Benedict s reagent were made daily on casual specimens of urine throughout observations Although many of the infants showed traces of reducing substances in their control urines before therapy these minimal amounts increased to 1 to 4 plus reactions in 20 of the 22 observations with ACTH administration In 7 the tests were interpreted as 3 to 4 plus and in 13 as 1 to 2 plus In contrast tests of the control urines were negative in 4 showed traces of reducing substances in 17 and in only one instance gave a 2 plus reaction Even in the last instance it became 3 plus in the test period Fermentation tests of the reducing substance in the urine identified it as glucose A return to pretreatment levels occurred in all of the infants by the second day of the postperiods

The glycosuria of premature infants with ACTH therapy is probably explained by the diversion of amino acids from protein synthesis to a more rapid conversion of these acids to glucogenetic moieties This mechanism has already been proven for older individuals

Although this gluconeogenetic effect of ACTH on amino acid metabolism is generally accepted as being mediated through stimulation of secretion of the 11-oxyadrenocortical steroids cortisone one of these oxysteroids failed to evoke glycosuria in eight observations Compound A another 11-oxysteroid was equally ineffective in another observation As might be anticipated this failure of a glycosuric response also held true for the eight observations with the other four 11 desoxysteroids DCA testosterone progesterone and Compound L

EFFECT ON EOSINOPHILS The circulating eosinophils also responded differently to the admini tration of ACTH and the adrenocortical steroids The iced eosin acetone method was used for counts on capillary blood

Four hours after the first dose of 3 125 to 12 5 mg of ACTH the eosino

phil count fell 50 per cent or more below pretreatment levels in only 3 of 19 infants A depression of this magnitude was however present in all of the infants by the end of 24 hours of divided therapy at 6 hour intervals By the end of 48 hours of treatment all the eosinophils had virtually disappeared from the peripheral blood The eosinopenic stimulus of ACTH may therefore be somewhat delayed in premature infants but their response is nevertheless maximal with continuing therapy Return to pretreatment levels following stoppage of injections was gradual Only one half of the infants reached or exceeded these values by 48 to 72 hours of the postperiods

Eosinopenia following cortisone was far less marked Only 3 of 8 infants showed a maximum drop of 50 per cent or more and in none of these did it reach 100 per cent even with total dosages of 700 mg Return to foreperiod values was in general more prolonged with this steroid than with ACTH the longer action probably being a function of its greater insolubility and more delayed absorption

No consistent or significant changes in eosinophil counts were observed with Kendall s Compound A 11-dehydrocorticosterone or with any of the other 11-desoxyadrenocortical steroids

CIRCULATORY EFFECTS Finally mention should be made of the striking vasomotor phenomena induced by hormonal administration

Twenty two of the 24 courses of ACTH therapy were accompanied by either generalized pallor or local blanching at the site of injection or by both

Systemic pallor of considerable intensity was exhibited in 19 of the 24 observations It appeared from three to thirty minutes following each injection and persisted in peak form for a matter of minutes or hours With continued treatment the infants assumed a constant grayish pale color which remained throughout the test periods and for 4 to 12 days after therapy was stopped

Localized blanching at the site of ACTH injections accompanied or followed systemic vasomotor reactions in a large majority of the infants The intensity of this local effect varied from one injection to the next in the same infant on the same dosage and with the same lot of ACTH When present it frequently persisted for as long as 8 to 12 hours following injections

Whether these vasomotor responses stemmed from the adrenocorticotropin itself or from contamination with posterior pituitary pressor or oxytocic principles contained in the ACTH preparations is not certain Manufacturer s assay revealed that both of these contaminants were present in trace amounts in the lots of ACTH which were employed in these observations Unfortunately blood pressures were not taken because of technical difficulties

In one of eight observations with cortisone local blanching of moderate degree was noticeable at the site of injection another infant exhibited gen-

eralized pallor No vasomotor effects were noted in the other six observations with cortisone nor with any of the five other adrenocortical steroids

The marked insolubility of Reichstein s Compound L led to residual small hard lumps at the sites of injection Two of these culminated in sterile abscesses which required incision and drainage

May I emphasize that the data presented in this report should be viewed in the light of pilot observations? Much more work needs to be done and many more infants studied over more prolonged periods to validate the suggestions embodied in this presentation Sufficient evidence has however emerged to indicate that the use of pituitary adrenocorticotropin and the adrenocortical steroids provides an excellent means for studying physiologic interrelationships and mechanisms in premature infants and that these hormones may have real promise in their clinical applications as they relate to the high neonatal mortality rate of premature infants

Professor BARNETT (New York) I have summarized observations on one of the infants included in Professor Levine s report which indicate the effect on balances of nitrogen sodium and potassium in one premature infant who received ACTH for a period of 7 days in dosages of 50 mg a day

On starting treatment a prompt negative nitrogen balance occurred This was followed after cessation of treatment by a marked positive balance in excess of pretreatment value

At the very beginning of treatment the infant went into negative sodium balance which again was compensated during the postperiod

The potassium balances were in most instances irregular During the first three days of treatment there was a negative balance of 1 4 mEq for 24 hours but during the last three days of treatment the potassium balance had again become positive to about the same extent as during the control period It was irregular in the postperiod

These observations tend to fit in with the rather marked loss of weight which the infants showed and which seems to be accounted for by loss of nitrogen and also by loss of extracellular fluid as reflected in the sodium balance

With premature infants who are on a more constant dietary intake and perhaps under more constant stress than are adults we may find more consistent effects of hormones than have been found in the treatment of adults

Dr LUFT (Stockholm) We have been working with a pure ACTH protein preparation (L1) in cases of rheumatoid arthritis in adults It is easy to keep such patients in a metabolic ward for a longer period of time

The main part of our work has consisted of balance studies with ACTH We have studied especially the effect of dosage and have been concerned with

fluid-electrolyte balances and with metabolic processes that are connected with nitrogen metabolism

We have found that the effect of ACTH on the electrolyte and water balances is dependent to a very marked extent on the dosage and the duration of treatment At all dosage levels of ACTH in the first three days of treatment sodium chloride and water retention resulted and this was followed by a very marked sodium and water excretion If we had stopped treatment at three days we would have had a marked retention of sodium chloride and water but by continuing for another three days we came back to the original balance However if the ACTH administration was continued for another week or two we obtained different balances for sodium chloride and water depending on the daily ACTH dose

With a daily ACTH dose of 3 to 6 mg a slight negative balance of sodium chloride and water occurred With higher dosages we obtained very marked sodium retention The small ACTH dosages gave insignificant changes in the potassium balance except for the first two days when there was a very marked excretion even during the first four hours of ACTH administration With larger doses of ACTH we obtained a very marked negative potassium balance during the period of administration

With high doses of ACTH and with this preparation a high dose level was about 12 to 15 mg daily we obtained a very marked loss of potassium and a retention of sodium of the same magnitude in milliequivalents When the values for the sodium and potassium balances were corrected for changes of extracellular fluid and of protein metabolism we still had a marked sodium retention and potassium loss The chloride balance did not show any significant changes

I am not able to say whether this sodium retention and potassium loss mean a shift of sodium and potassium between the extra and intracellular water compartments This may be the case however

The effect of ACTH on protein metabolism and allied metabolic processes was markedly different with small or large doses

With the small dose there was no significant effect on protein and carbohydrate metabolism But with an ACTH dose of about 12 to 15 mg per day we got a very marked effect which was not increased with a dose of ACTH over 13 mg a day There was an almost maximal effect at 13 mg per day except for the excretion of reducing substances where a linear response to the ACTH dose was seen

During administration of ACTH there is a loss of fluid With small doses of ACTH the loss seems to be limited to extracellular water and with larger

eralized pallor No vasomotor effects were noted in the other six observations with cortisone nor with any of the five other adrenocortical steroids

The marked insolubility of Reichstein s Compound L led to residual small hard lumps at the sites of injection Two of these culminated in sterile abscesses which required incision and drainage

May I emphasize that the data presented in this report should be viewed in the light of pilot observations? Much more work needs to be done and many more infants studied over more prolonged periods to validate the suggestions embodied in this presentation Sufficient evidence has however emerged to indicate that the use of pituitary adrenocorticotropin and the adrenocortical steroids provides an excellent means for studying physiologic interrelationships and mechanisms in premature infants and that these hormones may have real promise in their clinical applications as they relate to *the high neonatal mortality rate of premature infants*

Professor BARNETT (New York) I have summarized observations on one of the infants included in Professor Levine s report, which indicate the effect on balances of nitrogen sodium and potassium in one premature infant who received ACTH for a period of 7 days in dosages of 50 mg a day

On starting treatment a prompt negative nitrogen balance occurred This was followed after cessation of treatment by a marked positive balance in excess of pretreatment value

At the very beginning of treatment the infant went into negative sodium balance which again was compensated during the postperiod

The potassium balances were in most instances, irregular During the first three days of treatment there was a negative balance of 1 4 mEq for 24 hours but during the last three days of treatment the potassium balance had again become positive to about the same extent as during the control period It was irregular in the postperiod

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During administration of ACTH there is a loss of fluid With small doses of ACTH the loss seems to be limited to extracellular water and with larger

doses to intracellular water After withdrawal of ACTH there was a marked loss of water sodium and chloride that lasted for several days

As to the mental and emotional effects obtained in children similar changes occurred in our adults but euphoria is common in adult patients given ACTH We did not see that with cortisone

We have also studied the effect of DCA and cortisone in rheumatoid cases The effect of cortisone on the balances mentioned is to some extent the same as with ACTH The changes do not appear during the first days of treatment however but after 3 to 4 days

As to the carbohydrate metabolism *was there any correlation between the* values you got with the fermentation test and with the Benedict test? We have not found any correlation

Professor LEVINE (New York) Dr Luft found that the effects of *ACTH depended not only on the dosage but on the duration of administration* You will recall that no effect of ACTH on aromatic amino acid metabolism was obtained in premature infants unless ACTH was continued for 3 days or longer even when 100 mg a day was used Conversely with dosages as small as 12 5 mg a day continued for 5 days or longer the effect was obtained This too was an instance in which the effect of ACTH depended both on total dose and time

Since we merely made qualitative estimations of urinary sugar by the Benedict reaction and occasionally performed fermentation tests merely to demonstrate the presence of glucose we have no quantitative measurement of the amount of true glucose in the urine

Professor BARNETT (New York) It was formerly accepted in the United States that ACTH caused sodium excretion but the present literature in America on the question of electrolyte excretion needs clarification I was very interested to hear some of the factors that may be responsible for this It is also good to hear that large doses of ACTH do cause sodium retention and potassium loss because at least the electrolyte changes in Cushing s disease seem to be as we have known them before

You found no effect on protein metabolism with small doses of ACTH but *an effect appeared with doses of 12 to 15 mg which was not increased by* going up to as high as 50 mg a day You did not say specifically what these changes were and I should be interested in knowing whether they were nitrogen loss or retention

What effect did you *find ACTH had on the uric acid creatinine ratio?* Dr Shorr s group has some recent data to indicate that even this is irregular depending upon the previous uric acid creatinine ratio and they are becoming very dubious as to its value in judging the effect of ACTH

Dr LUFT (Stockholm) In our stud es there was n n r ased l to al n t ogen w th h gher doses—12 to 15 mg of ACTH and lso a lo crea ne ur c acid phosphorus and calc um Cre t n ne c r on d d n show any s nificant change

We d d not make any calculat ons on the ur ac d crea n n a o The crea n ne excret on was almost constant dur ng the control pe d he per t of ACTH administration and after withdra al of ACTH B e u a l excret on was always increased when we reached the ACTH h n creased the n rogen excret on and so of course th u n n ra o was ncreased

I was very interested to hear that one of the fa h trea ment in children is increase n appet te Ma ACTH about 3 to 6 mg a day the same benefi a the an anabol c effect on prote n metabol sm will o r arthri s pat ents e get a beneficial effect w th su

P ofessor LEVINE (New York) That is prec o do n the very small premature baby not v a t n bu giv ng m nu e doses of ACTH very early n l itam n C also I th nk that is possibly one of tl e on of th s study

P ofessor WALLGREN (Stockholm) Profes us when n fetal l fe the p tu tary gland might be exp c the vascular po nt of v e v?

Profes or RÄIHÄ (Hels nk) There s max m l n he ap lanes in the infund bular process of the p tu tary gland l en tl fc w l between 800 and 1500 gm and in pars d stal s hen tl e fetus e l s b n 1500 and 2500 gm (Table 46) We th nk th t nula on throu h l nervous sy tem of ACTH output develop in the l g of c n w the la e development of the connect on between th l r n n h p u ary Naturally th s does not mean that exogen u \ l H nn effect e

Le me add a word about the s ze of the adrenal du n f tal l fe We all know hat there s adrenal hypertrophy at b rth but th s hypertr phy is rel u ely greater the more immature the baby is It s at a m x n um n th th rd mon h of gestation (F g 205)

We have occas onally used testosterone in prematures if th y are l l l and ha e no s gns of d sease but are not ga n ng e ght We uch p matu e nfants small doses of testosterone one ten l of th adult do for f o x days I th nk e have had about 20 cases and u ually but not al ay the e ult has been a ga n n we ght n perhap t o th rd of our 20 ca

Professor LEVINE (New York) Our infants weighed between 1112 and 2240 gm at birth and at the time they were studied their weights varied between 1320 and 2500 gm As Professor Raiha implied we were giving ACTH so that we were replacing pituitary function Therefore the relation

TABLE 46
Increase of Capillaries in the Pituitary Gland with Growth of the Fetus

Weight of Fetus (grams)	Volume of Capillaries as Volume Per Cent of Tissue	
	Infundibular Process	Pars Distalis
120	0 4?	5 ?
800	0 44	9 1
1510	1 26	9 7
2480	—	17 2
2730	1 9?	—
3630	—	24 6

ship between the hypothalamic system and the anterior pituitary was not involved However the question that does arise is whether anterior pituitary hormones can mediate a response in small premature babies

I do not think that can be positively answered except to say that the re

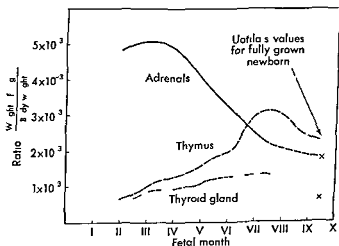


Fig 205 Development of the relative weights of the adrenals the thymus and the thyroid gland

sponse was precisely the same in the smallest baby as in the largest Apparently then that response is mediated when exogenous ACTH is given There have also been observations made on the excretion of 17 ketosteroids and the gluco corticoids in newborn babies Apparently the 17 ketosteroids were diminished in the 12 normal male newborn babies but the corticosteroids approximated the value per gram of adrenal tissue given for normal adults Perhaps anatomically and functionally newborn babies may have different adrenal glands from older individuals

You probably know of the great controversy concerning the value of testosterone I think that the majority of the people who have attempted to confirm Shelton s results in America have been unsuccessful

Professor BARNETT (New York) I would like to emphasize the last remark Professor Levine made Hardy and Wilkins in the *Journal of Pediatrics* (34 439 1949) reported that they failed to find any consistent increase in weight or other effects from giving large doses of testosterone to premature infants

Dr Luft have you any data relating to the observation of Conn and others on glycosuria at lower plasma glucose levels during ACTH therapy or an opinion about this?

I think some of us have been less impressed with the feeling of euphoria accompanying ACTH therapy at least in children than have others Certainly the children with nephrotic edema whom we treated felt terrible while they were getting ACTH I have the impression that this euphoria which was first noted in patients who were in pain and then were relieved may have arisen perhaps as a result of propaganda because people expected to feel better when they got ACTH It is difficult to tell how much of this is really a physiologic effect of the drug

Dr LUFT (Stockholm) We made observations similar to Conn s The fasting blood sugar in our patients during the control period was about 100 mg per cent With small doses of ACTH where we did not find any increase of urinary reducing substances the blood sugar did not rise either At the critical level of 12 5 to 15 mg a day there was a slight increase of the fasting blood sugar to about 120 mg per cent Simultaneously however a very marked increase of reducing substances appeared in the urine With our usual dextrose tolerance tests healthy subjects often have a blood sugar of about 175 mg per cent without sugar in the urine But during ACTH treatment with a blood sugar of about 130 mg per cent a very marked urinary excretion of reducing substances appears

Our patients have severe rheumatoid arthritis with pain They lose their pain thanks to the ACTH which makes them very happy

Professor LEVINE (New York) Have you experience with patients who do not have pain?

Dr LUFT (Stockholm) No we have only severe cases It is difficult for us to keep a patient in bed and give him the same food for three or four months unless he has severe pain

Professor WALLGREN *(Stockholm)* *Professor Levine could the loss* of weight that you have demontrated in ACTH treatment of premature babies be due in some degree to the hyperactivity and crying you so dramatically described in all these children? We know for example that when we feed young premature babies those fed by gavage gain more weight than infants fed the same amount by sucking

Professor LEVINE (New York) I agree that unquestionably part of the loss of weight is due to the increased activity of the infants during ACTH therapy There is also a negative nitrogen and sodium balance as Dr Barnett pointed out Both of these factors I think explain almost completely the loss of weight The metabolism probably rose not 100 per cent but perhaps 500 per cent with the marked activity and crying

Professor RÄIHÄ (Helsinki) I presume that these babies were in an oxygen incubator during these procedures

Professor LEVINE (New York) No they were all outside oxygen Ex*cept for a few we did not begin the study while they were in incubators*

Professor RÄIHÄ (Helsinki) Was there cyanosis in any of them?

Professor LEVINE (New York) We did not notice it but the pallor was so extreme that perhaps the constriction of the cutaneous capillaries may have interfered with the appearance of cyanosis even with an arterial oxygen unsaturation

Dr LICHTENSTEIN (Stockholm) We did some experiments with ACTH on pregnant rats in Cincinnati and we observed severe circulatory collapse a few minutes after injection We had several different preparations from Armour and Company some of which had a potency 20 per cent of *their standard while others were much higher* With the less potent preparations the impurities from the posterior pituitary were enough to explain this circulatory collapse

Dr BERGSTRAND (Stockholm) Our experience with ACTH on premature and full term infants is very limited and we have only been concerned with the eosinophils in circulating blood We started with the assumption which may be false that one can calculate the dose according to body weight or surface of the infants We began with about 0 43 mg per kilogram of body weight corresponding to Thorn s 25 mg for an adult We obtained the same results as Profe or Levine with little or no response in premature

babies to that dose during the first week of life We have doubled the dose with no response After several weeks when the weight had increased to about 2500 gm we have given the same dose and in a very few of the cases have seen a response

We have also given some injections to full term babies and there we have seen responses We have not calculated all the figures yet but the response though real is not a 50 per cent fall in eosinophils We have also studied the eosinophils the day before injection at the same times 8 and 12 o clock and the variations are generally very small If a decrease occurs it is not of the same degree as when ACTH is given

The reason that we started with small doses was partly that we had a very limited amount of ACTH and also that we were a little afraid apparently needlessly to use large doses We thought that since there was a rebuilding of the adrenal cortex during fetal life and early infancy there might be some risk in giving such large doses Can this eosinophil test be used in premature and full term newborn infants to discover adrenal insufficiency? We have not had the impression that the test is very reliable at this age

I think the literature on testosterone is not very convincing at the moment I made some experiments with testosterone on rabbits During the first weeks of life they were given testosterone with no difference in weight between the control group and the test group There was slight decrease in the length of the femur and tibia in the treated animals but no effect on the development of the ossification centers was seen by X ray

Professor LEVINE (New York) We had the same hesitation about using large doses of ACTH and so we had conferences with Dr Mote and a large number of the people who were working with ACTH on adults They universally said that we would have to use relatively larger doses with premature infants than with adults or older children

All of these infants left the hospital in fine hape and in follow up studies they continued to thrive without showing any evidence of either hyper or hypoadrenal function So at least with these short term observations giving large dosages was not harmful but I cannot say that they were beneficial

We think as you do that the eosinophil test particularly for prematures is not as good an index of the ACTH pituitary–adrenal cortex mechanism as in an older individual That impression is confirmed by the recent publication by White and Sutton (*Pediatrics* 5 376 1950) who state that with few exceptions premature infants generally fail to show a normal response to the epinephrine eosinophil depression test indicating an immaturity in [illegible] sistance to stress as mediated by the anterior lobe of [illegible] itary g[illegible] adrenal cortex

As was pointed out with regard to testosterone it is our impression that its use as a clinical method for improving the condition of premature infants is not established as a good procedure

Professor RÄIHÄ (Helsinki) Is there any information on the effect of ACTH on the lymphocytes?

Professor LEVINE (New York) I thought that question was going to be asked We wanted to study all of the blood elements including lymphocytes and leucocytes Dr Bierman who was doing the work was so overwhelmed with many of the other features that I think he did lymphocyte counts only in a few cases The results were not nearly as consistent as with eosinophils so that we did not continue that phase of the study

Dr LUFT (Stockholm) We followed the lymphocyte count in our cases The statistical evaluation did not show any significant changes from the control period

Dr Bergstrand asked an important question even for those of us working with adults Is the ACTH test so valuable? I should like to ask if the test is always significant in adults Our experience with the test is limited to about 100 patients with different endocrine disorders Of course in every case of Addison s disease where the diagnosis was already certain before the ACTH test was made we never saw a response in the eosinophils But we are not certain of the value of the test in the group of borderline cases where the ACTH test is supposed to be of the greatest value For example in the past week we had a patient with diabetes mellitus who showed no response at all during two ACTH tests We could not find any other signs of adrenocortical insufficiency We performed two more ACTH tests and at the third test she showed a normal eosinophil response

We have used testosterone propionate and methyl testosterone for the last 4 to 5 years in the treatment of disturbed somatic development in children We have the impression that we have obtained some improvement in these subjects in that they grow a little more rapidly But after seeing these patients two years later when they were more mature we have a feeling that they might have grown the same amount even without the treatment It may be of some importance that we make these children grow a little more rapidly during that period when the other children of the same age are growing But we have an impression that testosterone has little to do with the final results

Professor BARNETT (New York) We have exactly the same impression about testosterone in older children And I actually think that both Nathan Talbot and Moss and Wilkins would agree that it does not influence ultimate growth but only accelerates growth which may cause distinct pyschological harm

CHAPTER X

Panel on Metabolism in Newborn Infants

DISCRETE KIDNEY FUNCTION IN YOUNG INFANTS THE RATE OF POSTNATAL DEVELOPMENT

Professor BARNETT (New York) We have been interested in the status of kidney function and its rate of development after birth from three general points of view First as pediatricians we should like to know whether a comparatively decreased renal function handicaps the young infant in his physiologic adjustment and if so whether the rate of development can be accelerated or if specific measures are needed in disease to compensate for the decreased function

Second we are interested in whether certain types of kidney disease in older infants and children may be the result of the failure of normal functional development of the kidney

Third as physiologists we are interested in the developmental aspects of kidney function in young infants to attain an increased understanding of renal physiology as a whole

For these reasons we need to know how function in young infants differs qualitatively and quantitatively from that in the mature subject I should like therefore to consider the present status of our knowledge of kidney function in young infants

It was about ten years ago that for a variety of reasons interest was aroused in kidney function in very young infants McCance in England had been impressed with the difficulty of obtaining satisfactory excretory pyelograms in very young infants and this suggested to him that kidney function might be decreased Professor Levine Dr Gordon and others at Cornell had ob

served that when premature infants were changed from human milk to cow's milk with a higher sodium chloride content there was a lag in the excretion of the excess sodium chloride suggesting that kidney function might not be fully developed

At Washington University in St Louis we had been impressed by a report of Greenwald and Popper on the histologic characteristics of the glomerulus of the young infant which showed that instead of a basement membrane surrounding the capillary tuft in the glomerulus there was actually high columnar epithelium It looked to them trying to read function from structure as if filtration could not occur as readily as it could across a basement membrane In addition the capillary tuft was small and collapsed instead of being expanded and occupying most of the space

In 1940 the techniques for investigating kidney function that had been recently developed by the kidney physiologists demanded the quantitative collection of urine which in newborn infants was a difficult and hazardous procedure We therefore attempted to estimate the rate of glomerular filtration without collecting urine using inulin clearance as measured from the slope of the line representing the disappearance of inulin from the blood after a single injection We related the slope of this line to actually determined clearances at different levels of clearance and found that full term newborn infants had inulin clearances of only 30 to 40 per cent of normal This method of estimating glomerular filtration rate has been more extensively investigated since then but we have not thought it suitable for quantitative work of the type we wanted to do After the war with the availability of effective antibiotics which could prevent urinary bladder infection we felt justified in catheterizing small female infants to determine clearances directly

Therefore our present methods of investigation of kidney functions in young infants include the standard techniques that are applied to adults We give a constant intravenous infusion during the equilibrium period and during the clearance period we collect urine by catheterization washing out the bladder with sterile water and air We draw venous blood samples in small amounts for the determination of the concentrations of the various substances in serum

Before we could begin it was necessary to establish that the substances used in adults would be handled in the same way by the kidney of the young infant and we were particularly interested in knowing whether inulin clearance was at the level of glomerular filtration in young infants

The criteria that had been set up by Homer Smith and his school for substances being cleared at the level of glomerular filtration included first that the clearance be independent of the plasma level over a wide range We were

able to demonstrate in premature infants that the clearance of inulin was independent of the concentrations of inulin in serum ranging from 12 to 150 mg per 100 ml Second the clearance of the substance should be independent of the rate of urine flow and this was demonstrated to be true in premature infants Third the clearance of a substance measuring glomerular filtration rate ought not to be influenced by the simultaneous administration of other substances We were able to show that the clearance of inulin was uninfluenced by mannitol para aminohippurate (PAH) thiosulphate glucose and penicillin Finally a substance which measures glomerular filtration rate ought to have an identical clearance with other substances known to

FIGURE 206

Renal Functions in Premature Infants (Barnett et al)

	GLOMERULAR FILTRATION RATE (INULIN CLEARANCE) ml per min	RENAL PLASMA FLOW (PAH CLEARANCE) ml per min	MAXIMUM TUBULAR EXCRETION (Tm_{PAH}) ml per min	FILTRATION FRACTION (C_{IN}/C_{PAH})
Premature Infants actual values	4 65	14 6	1 26	0 35
Premature Infants values per 1 73 m	46 5	146	12 6	
Adult Values per 1 73 m	±123 18	±634 125	±78 9 13	0 20
Premature Infants per cent of adult value per 1 73 m²	38	23	16	

159 observations on 27 premature infants Age—28 days
Wt —2 40 kg
S A —0 18 m²

measure glomerular filtration rate We were able to show that inulin clearances in premature infants were identical with those of thiosulphate and endogenous creatinine and that it had the same relation to mannitol clearance in premature infants as it had in adults

We believed therefore that we had fairly firm evidence that inulin clearance measured glomerular filtration

Values attained for inulin clearance in a series of premature infants are shown in Figure 206 The clearance of inulin was found to be 4 65 ml The correction factor for comparing these with adults on the basis of surface area is 10 since the average surface area is about 1 73 sq m in the adult Thus these values correspond to 46 5 ml per minute per 1 73 sq m instead of the normal adult value of 120 to 130 ml per minute per 1 73 sq m

We wished next to measure renal plasma flow with the expectation that it

would be less reduced than the inulin clearance For if the low inulin clearance were due to a mechanical hindrance to filtration from the columnar epithelium one would have expected to find a low filtration fraction However when the clearance of PAH was measured we found an even greater reduction in renal plasma flow than in inulin clearance with a consequent abnormally high filtration fraction

Equating clearance of PAH with renal plasma flow however has to be done with caution because such an equation depends upon all or practically all of the PAH being removed from arterial blood in one passage through the kidney This has been demonstrated to be the case in the normal adult kidney by renal vein catheterization but it has not been demonstrated in small infants We shall therefore not know if this is a true value of renal plasma flow until extraction ratios are measured

The next important step in the development of this subject was the determination of the rate at which kidney function increased to the adult values If one corrects mannitol clearance to a standard surface area the adult levels are reached somewhere between the second and tenth months

The selection of a standard of reference in interpreting figures such as these is a very difficult problem If one uses weight or body water as suggested recently by McCance instead of surface area one finds that per kilogram of body weight or per liter of body water the inulin clearance of the newborn infant is practically within the normal range for adults However if weight or body water is used as a standard of reference at about two to five years of age the inulin clearance becomes 200 or 300 per cent of that of the adult McCance believes that the latter values may not be unphysiologic when one considers the total metabolism The proper standard of reference must remain at present therefore an unsettled question

The premature infant as was mentioned in the talks on digestion affords a situation in which one can attempt to study the effect of function on maturational growth under fairly normal conditions It is true that there is evidence for prenatal kidney function but the extent of this function is so small compared with postnatal requirements that it can almost be disregarded So if one compares the renal function of a group of infants who have been in the uterus during the last 60 days of fetal life with a premature group whose kidneys have been functioning during these same 60 days one can attempt to evaluate the effect of this postnatal function on maturational growth Such an attempt was made by comparing one group of premature infants whose birth weight ranged from 2 to 2 5 kg but whose age was less than 2 weeks with a second group of infants of equivalent weights whose ages ranged from 50 to over 100 days If one accepts the limitation of weight as a measure of

gestational age the difference between these two groups is so great that it is clear they repre ent different infants

Figure 207 shows that inulin mannitol urea and PAH clearances and maximum tubular excretory capacity of PAH are higher in the more premature infants of approximately the same gestational age We have not as yet been able to compare these values with similar results in full term infants but what information is available indicates that one would expect a greater increa e in function after two months of extrauterine life than is shown here These results suggest that although function is somewhat increased in the group of infants who have been using their kidneys there may be a limiting

FIGURE 207

Renal Clearances in Premature Infants*

NO	DAY OF OBSERVATION			BIRTH WT kg	CLEARANCE ml m n				TM, mg m n	CIN / C u
	AGE days	WT kg	HT cm		INULIN	MANNITOL	UREA	PAH		
8	3–13	2 1 2 4	46–47 5	1 2 5						
	Mean Uncorrected				4 7	4 2	2 9	14 6	1 3	34
	Range Uncor ected				3 3–6 6	3 1–6 1	1 8 4 1	9 9–16 5	) 6–2 3	27 42
	MEAN PER 1 73 M				48	43	30	149	12 9	
9	19 107	' 2 2 5	43 5–47	0 7 1 4						
	Mean Uncorrected				6 9	5 8	4 5	20 6	2 2	34
	Range Unco ected				5 1 11 1	1–6 8	' –8 0	12 8 35 0	1 2 5 3	29– 42
	MEAN PER 1 73 M				6	58	44	201	20 8	

Barnett H L K dney Function in Young Infants" *Ped i c* 5 176 1950 Charles C Thomas Publisher

factor in infants under 2500 gm which prevents as rapid an increase as one might expect from the rate of development in full term infants

It is of course true that the kidneys in premature infants do a remarkably creditable job This however is under normal circumstances and it is possible that the low level of bicarbonate and high level of chloride in the serum of premature infants may reflect the immaturity of their kidney function Support is lent to this by the observation that premature infants fed protein milk with a great excess of chloride over sodium developed very severe acidosis whereas full term infants fed the same milk did not develop acidosis

Figure 208 shows the results of an attempt by Gordon and his associates to measure the capacity of the kidney of the premature infant to form ammonia

and conserve sodium in the presence of an acid load Although there is overlapping of the data and more data are needed the full term infants who were given ammonium chloride were able to conserve sodium by substituting ammonia for sodium to a somewhat greater extent than the premature infants

A practical application of this knowledge lies in the very important area of calculation of drug dosages in young infants I think we have not paid

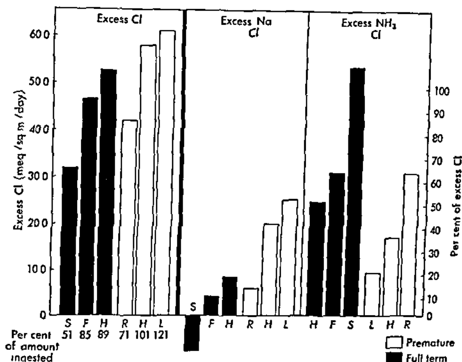


Fig 208 Cl Na and NH excretion during NH Cl ingestion in young infants (Gordon H H McNamara H and Benjamin H R "The Response of Young Infants to Ingestion of Ammonium Chloride" *Pediatrics* 2 290 1948 Charles C Thomas Publisher)

sufficient attention to the decreased kidney function of young infants particularly in calculating the dosage of drugs which may be toxic and whose main route of excretion is renal This has not been extensively explored but we have some data obtained with penicillin which demonstrate what may be expected with other drugs *The results shown in Figure 209 indicate that* per unit of surface area the clearance of penicillin in premature infants is much lower than in children from 3 to 12 years of age This is even true if calculated per unit of body weight as drug dosages usually are Clearly

we need to know more concerning the relative rates of renal excretion of drugs in young infants

FIGURE 209

Clearances of Inulin and Penicillin G*

No	Age	Wt kg	Ht. cm	Mean Clearances ml per min per 1 73 m² Inulin	Penicillin G
			CHILDREN		
12	3-12 yrs	13-46	95-150	127 (93-145)	560 (311 934)
			PREMATURE INFANTS		
4	4-8 days	2 2 2 3	47-47 5	44 (31 53)	90 (72 114)

Barnett H L Kidney Function in Young Infants *Pediatrics* 5 178 1950 Charles C Thomas Publisher

RENAL FUNCTION DETERMINED BY A NEW TEST

Dr STRÖM (Stockholm) Serum inorganic phosphate which in children amounts to 5 0 to 6 5 mg per 100 ml varies with the utilization of carbohydrate This is the result of the binding of phosphoric acid to carbohydrate to form hexose phosphates which play an important role in carbohydrate metabolism Increases in serum phosphate occur with disease of the kidneys and are signs of renal insufficiency The cause of this phosphate retention cannot be definitely stated The diminished excretion of phosphoric acid by the kidneys with corresponding increases in blood and tissue levels is an important factor in the development of acidosis in cases of renal insufficiency Thus it is understandable that poor kidney function can be a dangerous complication in diabetic acidosis

McCance has investigated the titratable acidity and phosphate excretion in infancy He points out that the paucity of phosphates in many infants urines must be a handicap to the infant in excreting acid It has in fact often been argued whether the newborn infant is or is not in a state of acidosis At any rate infants may be badly equipped as far as their kidneys are concerned for dealing with acidosis

Hahn Hevesy and Lundsgaard (1937) demonstrated in studies with rabbits that parenterally administered radioactive phosphorus is partly stored in the organism and partly excreted After approximately four weeks 45 per cent had been excreted in the urine and 11 5 per cent in the feces Moller strom studied the earliest time that radioactive phosphorus appeared in the urine after oral administration of P With prior administration of glucose phosphate appeared in the blood within 10 minutes predominantly in organic

form In the urine the excretion likewise began after 10 minutes and at the end of the first hour the isotope excretion had risen to considerable levels In human subjects Govaerts and Lambrechts found that 2 53 per cent of the P^3 injected intravenously was eliminated in the urine in four hours Exogenous phosphorus is treated differently from endogenous phosphorus in the organism Govaerts found that injected inorganic phosphorus is excreted more rapidly than the normal plasma phosphate Part of the plasma phosphorus may exist in another physical-chemical form which Govaerts calls *phosphate X*

There may be exchange of phosphorus between the inorganic form and phosphate X which does not occur in vitro The renal threshold for phosphorus is due to renal factors hormonal factors and to the physical-chemical state of plasma phosphorus

Ostling has given P^3 intravenously to persons with diseases of the urinary tract Separate urine samples were taken from each kidney at the same time by catheterization and showed different P /P^{31} ratios in persons with disorders of one kidney These results support Govaerts conclusion that endogenous and exogenous phosphorus are handled differently by the kidneys

Tweedy and his associates found that parathyroidectomized rats given P^{32} *excreted none in the urine When these rats received parathyroid hormone* however P^{32} appeared in the urine Harrison found that parathyroid hormone decreases the rate of the reabsorption of phosphate by the renal tubules and lowers the concentration of phosphate in the plasma

In investigations on diabetics at the Wenner Gren Institute in Stockholm it was observed that the excretion curves of phosphorus of a number of patients deviated noticeably from the majority These patients were found to have renal damage These results suggested that radioactive phosphorus might through measurement of phosphorus excretion be clinically applicable as a kidney function test

Therefore I made some studies on phosphorus excretion in children The experiments were carried out as follows 300 to 500 ml of water depending on the age of the child were introduced into the fasting stomach the urinary bladder was emptied and the radioactive phosphorus was injected Blood specimens were taken after relatively short intervals and with a suitable amount of radioactive phosphorus (approximately 50 microcuries) it was convenient to use a capillary blood sample of 0 4 ml The urine samples were taken if possible simultaneously *with the blood*

For the determination of phosphate the Fiske Subbarow method as modified by Brigg and Lindberg was employed The radioactivity of the specimens was measured in the usual way in a Geiger Muller counting cham

ber The specific activity i e the relation between counts per unit volume and the total phosphorus in milligrams in the same unit volume was calculated It was shown that normally the specific activity in urine was significantly greater than in blood The greater portion of the activity in the blood usually disappeared within the first two hours while urinary excretion was very high in the same period

In patients with damaged kidneys especially acute nephritis it appeared that the excretion of the organically bound phosphorus as well as the inorganic phosphorus was greatly diminished With improvement of the clinical symptoms the excretion of phosphorus was increased

I have used this method with slight modifications in a group of premature children weighing between 1020 and 2660 gm They were born 2 to 9 weeks prematurely and the test was performed within 9 days after birth They were all healthy and further development was quite normal

The injection was made intraperitoneally and about 10 microcuries per kilogram of body weight were given Urine was collected by catheterization

The specific activity was lower in the urine than in the blood and the flat excretion curve obtained corresponds to that seen in patients with damaged kidneys One might conclude that the curve of premature infants corresponds to subnormal kidney function or perhaps to not yet fully developed function

It seemed remarkable that the specific activity of the urine was so low compared to the blood Three possibilities for this may be discussed

1 It is difficult to empty the bladder completely and the low activity might be due to this This seems the most likely explanation

2 There may be diffusion through the cell membrane of the tubular epithelium

3 There may be production of a phosphate fraction in the kidney which is not labeled The fractions which are not labeled are lipoid phosphorus and the nucleophosphorus But a simple calculation shows that practically all the phosphorus of the organism is labeled very soon

Dr LUFT (Stockholm) In our cases of adrenocortical insufficiency we have observed a decreased glomerular filtration rate and renal plasma flow We have been able to obtain normal values after treating these patients with adrenocortical hormones

Is it possible that premature children may have a disturbed adrenocortical function? We have found that administration of an adrenal steroid like DCA sometimes increases renal phosphate excretion and in one case it also caused a decrease of inorganic plasma phosphate

Dr JOSEPHSON (Stockholm) As Professor Barnett pointed out if one

wants to determine the renal plasma flow by PAH one must be sure that the extraction is complete and we do not know that this is true in small children *and especially in prematures* What was the concentration of PAH in the plasma in these experiments? It has to be low enough of course so that one is not above the depression limit (Tm) We know in adults that about 90 per cent of the PAH is extracted if the concentration of the PAH in the plasma is kept below about 10 mg per 100 ml With the concentration above this limit complete extraction is not reached and the clearance of PAH is not a measure of the renal plasma flow

I do not think this question can be completely solved without renal vein catheterization which may be very difficult to carry out in small children However I should like to mention some data we have obtained in adults

We wished to determine where the depression limit is in healthy persons and also in patients with renal disease We wanted to know if and when the methods for determining renal plasma flow were applicable in patients with kidney disease

We carried out a series of what we call staircase experiments We gave the subject a sustaining infusion of PAH intravenously and increased the concentration of PAH gradually in steps in order to study the extraction at different levels of concentration The extraction was determined by renal vein catheterization Experiments of this kind without renal vein catheterization were carried out earlier by Smith and co workers in what they called titration experiments but without knowledge of the concentration of PAH in the renal vein plasma such experiments are not definitive

In a patient who had almost completely recovered from a mild case of acute glomerulonephritis there was 90 per cent extraction of PAH at a plasma level of 3 mg per 100 ml or less Furthermore the extraction remained constant as the concentration of PAH was increased until a level of 12 to 15 mg per 100 ml was reached

On the other hand in a patient suffering from acute glomerulonephritis at the time the test was performed extraction of PAH was only about 60 per cent *even with a* PAH concentration below 2 mg per 100 ml and it fell even further as the PAH concentration was increased

Thus in the latter instance it was impossible to measure renal plasma flow by PAH clearance I wonder whether this is not similar to what will be found in very young infants

There is another way to approach the problem without renal vein catheterization and that is to raise the plasma level of PAH gradually and note where the PAH clearance starts falling I should be very interested to know if you have done that in these premature children

As far as I understand the technique of maintaining a constant infusion and catheterizing these small children must be very difficult Therefore another possibility may be of interest which we have not yet tried in children We have tested it in adults however and it has turned out very well

If a single injection of a solute for instance inulin or PAH is given intravenously a curve of plasma concentration versus time can be obtained If all the urine that is excreted is collected until everything injected has been recovered the clearance for the corresponding time can be calculated by dividing the excreted amount by the area under the plasma concentration curve In fact if the solute given is known to be 100 per cent excreted in a given time even urine collections are unnecessary This might be a simple way to carry out clearance experiments in small children In adults we have made comparisons between this method and the ordinary clearance method and have found very good agreement

Professor BARNETT (New York) We have measured kidney functions in an infant with marked adrenal insufficiency during the first few weeks of life and have found values that were within the normal range However lowering of glomerular filtration rate in adrenal insufficiency certainly has been a consistent finding in both experimental animals and in patients I am interested to hear that it can be brought back to normal by giving adrenal hormones In many of the reported instances it could not be completely corrected just with adrenocortical hormones

The whole subject of endocrine influences on kidney function is an important one and particularly the evidence that there is some direct pituitary influence There is for example the amazing demonstration that in the absence of the anterior pituitary a single kidney remaining after unilateral nephrectomy fails to hypertrophy

In answer to Dr Josephson the concentration of PAH during our clearance studies was always under 2 mg per 100 ml and was frequently around 1 mg per 100 ml If one judges from measured values for Tm one would expect complete extraction at this low level which only requires about one fifth of the ability of the kidney to excrete PAH However this is very indirect evidence

In staircase observations without renal vein catheterization is the depression in the clearance of PAH with increasing plasma level different enough when the initial extraction is complete from when it is incomplete to be certain of when there is incomplete extraction at low plasma levels? Could you have differentiated between the two patients you described without having done renal vein catheterization? If a gradual fall in clearance were found in our premature infants it would suggest that extraction was not complete initially but would not prove it

Dr JOSEPHSON (Stockholm) What I meant was that if the concentration of PAH in the plasma is kept at say 1 mg per 100 ml and then increased to about 2 mg per 100 ml and the PAH clearance remains constant that is admittedly not proof but it is some evidence that the depression level has not been reached That might be possible to do in young children

One point which may be of interest in this discussion of endocrine influences on the kidney came out of a series of experiments on tubular function Two months after thyroidectomy in young rabbits their tubular secretory capacity was practically zero

Professor BARNETT (New York) The published results of Dr Tudvad in Copenhagen I think have contributed a great deal to this field They have

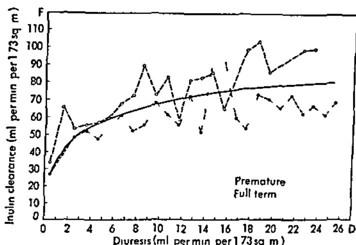


Fig 210 Inulin clearance in relation to diuresis in premature and full term babies

demonstrated for instance some very interesting emotional effects on kidney function in infants

Dr ROTHE MEYER (Copenhagen) Dr Tudvad was not able to attend this panel Figure 210 shows some results of his work The glomerular filtration was determined by inulin clearance in 14 premature infants aged 1 to 203 days and in 7 full term infants aged 9 to 150 days In each infant a series of 6 to 15 continuous clearance estimations were made a total of 408 clearance determinations The inulin clearance and diuresis showed marked individual variations With increasing diuresis there is an increase of inulin clearance The clearance of the full term infants was higher than that of the prematures It was thought that this latter finding might be related to the fact that most of the full term babies were fed on half skimmed citric acid milk while the majority of the prematures were fed on human milk

In order to clarify this point premature infants weighing 2000 to 2500 gm were selected (in order to avoid the influence of birth weight) and within this group infants aged 22 to 103 days were selected because during this period the inulin clearance was almost constant Now these infants were subdivided into those fed cow s milk mixture and those fed human milk there being a total of 142 clearance periods among them The inulin clearance showed the same rise with increasing diuresis as noted before Furthermore the inulin clearances of the infants fed cow s milk were higher than those of the infants fed human milk indicating that a high protein feeding results in a higher glomerular filtration

The two facts Dr Tudvad wanted to point out then were (1) inulin clearance rises with increasing diuresis and (2) the glomerular filtration rate is higher on high protein feedings

MECHANISMS INVOLVED IN TETANY OF THE NEWBORN

Professor BARNETT (New York) I recall that in the discussion of vitamin D Professor Nicolaysen at one point asked What is rickets? He at least was able to give a definition of his own If I were asked What is tetany in the newborn? I am afraid I could not give any definition Actually I shall not be discussing tetany of the newborn so much as the phase of it that concerns renal phosphate excretion What is called tetany of the newborn is a condition in which a group of symptoms appear the most outstanding of which is increased neuromuscular irritability in the newborn infant associated with changes in the calcium and phosphorus concentration in the blood By most people s definition it is required that either the calcium concentration be low or the phosphorus concentration be high or that there be a reversal of the usual relation between the two Our interest was in the role that phosphorus plays in these relationships

It is generally accepted that there exists an over all reciprocal relationship between the calcium and phosphorus concentrations in the blood and that one can induce a hypocalcemic tetany in dogs by infusion of concentrated solutions of inorganic phosphate If one accepts the assumption that a high inorganic phosphorus concentration in serum may in itself lead to a low concentration of serum calcium and thus to symptoms of tetany it becomes of interest to know what factors may produce so-called hyperphosphatemia in newborn infants

I should point out that insofar as these chemical changes are related to the disease we have observed and it has been reported many times that one may see an infant with given levels of serum inorganic phosphorus and serum

calcium with no signs of tetany and another infant with exactly the same levels showing active tetany I think therefore that the relation of the chemical changes to the symptoms is far more complicated than the mere concentrations of these two substances in the blood It has been recently shown in experimental animals for instance that at a given low level of serum calcium and serum potassium there may be no signs of neuromuscular hyper irritability however with the calcium remaining the same an elevation of the potassium will result in convulsions Thus many factors are concerned with this syndrome

I should like to limit our attention to mechanisms which may be responsible for hyperphosphatemia in young infants Table 47 lists those mechanisms that may be involved in tetany of the newborn

TABLE 47

Possible Mechanisms Involved in Tetany of the Newborn

(1) High phosphorus (P) intake—all infants on cow s milk
(2) Low glomerular filtration rate (GFR) in all infants
(3) Moderate elevation of serum phosphorus (SP) in all infants
(4) Renal P excretion insufficient to lower SP because of
 (a) Transitory hypoparathyroidism?
 (b) Low GFR
(5) Greater elevation of SP in some infants because of
 (a) More marked hypoparathyroidism
 (b) Greater decrease in GFR
(6) Reciprocal lowering of serum calcium in all infants most marked in (5)
(7) Tetany in some infants of (6)

It is true that in relation to surface area kidney function or anything that one wants to use as a standard reference all infants receive a high phosphorus intake This is accentuated in infants fed cow s milk which some people have offered as an explanation for the apparently higher incidence of this syndrome in the United States It is also fairly well established that in relation to surface area basal metabolic rate or other standards of reference all newborn infants have a comparatively low rate of glomerular filtration as we have already discussed today In comparison with adult values there is a moderate elevation of serum phosphorus in all infants If the reason for this is a renal excretion of phosphorus that is insufficient to lower serum phosphorus this could be due either to a hypoparathyroidism or to the low rate of glomerular filtration Finally a reciprocal lowering of serum calcium may result most marked in infants with the highest levels of serum phosphorus which may then be followed by symptoms of tetany in some infants

The common explanation of the primary cause of the elevation of serum phosphorus at least in the American literature of the last ten years has been that a transitory hypoparathyroidism exists If a high intake of phosphorus such as supplied by cow s milk is combined with a more marked degree of hypoparathyroidism in some infants this may lead to sufficient elevation of serum phosphorus to produce symptoms and signs of tetany in the newborn infant It appeared however that not enough attention had been paid to the role of the lower glomerular filtration rate in infants who showed an endogenous elevation of serum phosphorus Since parathyroid hormone cannot be measured in blood directly in young infants both of these functions can be studied by determining the renal excretion of phosphorus

From our present knowledge of the effect of parathyroid extract on phos phorus excretion by the kidney we should expect that if the decreased renal excretion of phosphorus were due to hypoparathyroidism we should find that in infants with high serum phosphorus there is a greater reabsorption of phos phorus by the tubules If the primary cause of the decreased excretion is a low glomerular filtration rate then we would expect to find a low rate in infants showing hyperphosphatemia with either no change in the amount reabsorbed or a reduced amount reabsorbed

In order to study this we made observations during the newborn period on infants whose level of inorganic phosphorus was below 8 mg per 100 ml These were infants without signs of tetany all fed on cow s milk and con sidered to be normal in regard to the level of their serum phosphorus

In order to see how such infants respond to elevation of their serum phos phate through the range that is encountered in infants who show tetany single feedings of disodium phosphate were given by gavage and their blood and urine examined subsequently It was observed as shown in Figure 211 that as serum inorganic phosphorus increased there was the expected rise in phosphorus filtered calculated from the product of the phosphorus concen tration in the serum and the rate of glomerular filtration measured by clearance of inulin The phosphorus excreted was found to parallel the phosphorus filtered with the increasing concentrations in the serum There was very little if any change in the amount of phosphorus reabsorbed Thus there appears to be a Tm for reabsorption of phosphate above which phosphate is not reabsorbed even when more is presented to the tubules by greater filtration It also appears that newborn infants receiving cow's milk are operating at approximately their maximum capacity for tubular [illegible] of phosphate

With these data as a background we were interested in kno[illegible] th[illegible] infants with high endogenous levels of serum phosphorus [illegible]

less, reabsorbing the same and therefore excreting less or whether they were filtering the same reabsorbing more and therefore excreting less

Figure 212 shows values that were obtained in seven infants who had endogenous elevation of the serum phosphorus *We found that in relation* to the normal product observed in Figure 211 these infants were filtering phos

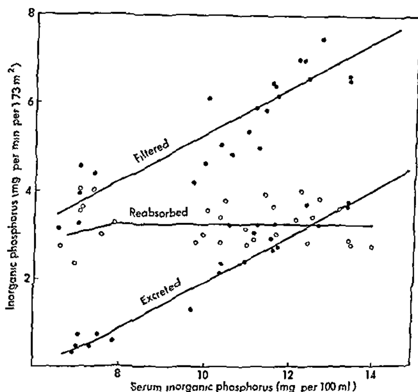


Fig 211 Phosphorus filtered (FP) excreted (EP) and reabsorbed (RP) in relation to serum phosphorus (SP) concentrations in 6 normal infants at endogenous SP levels and at levels elevated by ingestion of phosphorus The lines were fitted by free hand from points obtained by class averages

phorus within the same range of values as were the normal infants, but they were doing this at a higher serum concentration We found that they were also excreting the same amount but they were doing this too at a higher serum concentration Therefore, the rate of reabsorption was within the same range as that observed in the normal infants

These data could only be explained by a reduction in the glomerular filtration rate in these infants which in a sense required them to maintain

their serum phosphorus at a level which would permit them to filter the same amount and therefore excrete the same amount as a normal infant

In Figure 213 there is shown a comparison between the glomerular filtration rate of this group of infants and that of normal infants It is obvious that more data are needed but the clearances of inulin plotted against surface area of these seven infants are all below the mean of a group of normal infants who were studied in a similar way

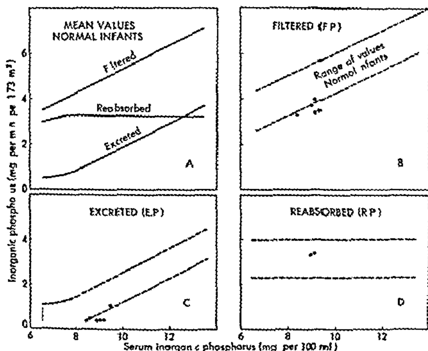


Fig 212 Phosphorus filtered excreted and reabsorbed at normal and high serum phosphorus levels in normal infants and in tetany of newborn

This suggests then that the primary cause of a greater elevation of serum phosphate in some babies is a greater reduction in glomerular filtration rate rather than a greater reabsorption of phosphorus in the tubules

That the newborn infants are capable of responding to parathormone is shown by Figure 214 With a fairly constant clearance of inulin the effect of 15 units of parathormone given intravenously on the ratio of the phosphorus to the inulin clearance (or on the per cent of filtered phosphorus which is excreted) is shown to be a very prompt and marked one and indicates that

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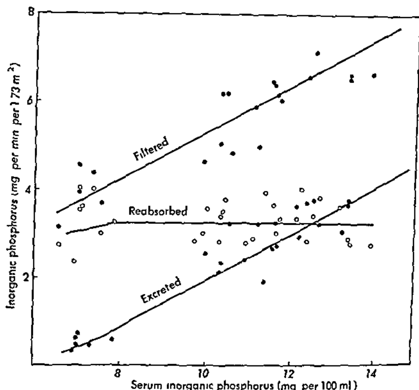


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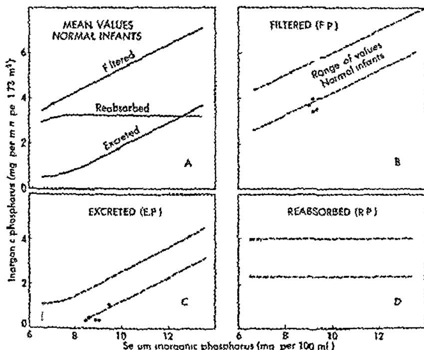


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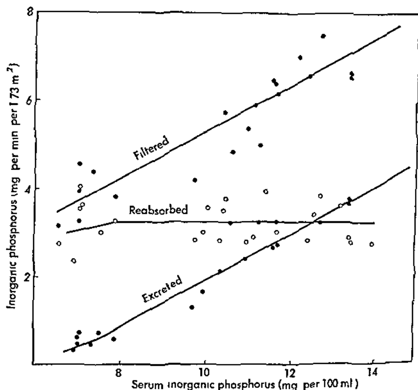


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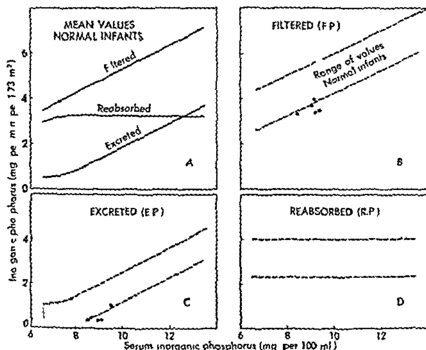


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the increased phosphorus excretion following parathormone is due to de creased reabsorption of phosphate since the rate of glomerular filtration does not change

This then would indicate that the failure of infants with high levels of serum phosphorus to reabsorb less phosphorus is not due to inability of the tubules to respond to parathormone The fact that such infants do have some

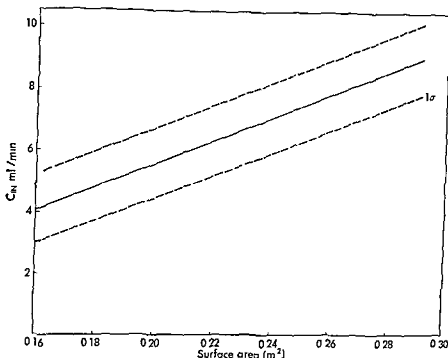


Fig 213 Values for glomerular filtration rate (C_{IN}) in nine observations on seven infants with hyperphosphatemia compared with values in normal infants *The solid line was fitted by the method of least squares to the data for* normal infants

parathyroid activity and could reabsorb more phosphorus is shown in Figure 215 These are observations made on the same infant on two different occa sions when the clearance of inulin was close to 4 5 ml per minute After the control observations shown on the left the infant was given aluminum hydroxide gel for a period of five days which effectively prevented intestinal absorption of phosphorus At the same level of glomerular filtration and the same concentration of phosphorus in the serum as during the control period the infant was excreting practically no phosphorus whatsoever Somehow the tubules of this baby knew that he had not been getting any phosphorus

for five days and needed it However it was clearly not the level of serum phosphorus that told him this This brings up the question of whether para thyroid excretion is determined not by the level of serum phosphorus but by the cellular composition of the gland or whether enzyme systems in the kidney tubules dependent on phosphorus concentration are affected independent of hormonal control

I leave the data then in a highly unsatisfactory state where they provoke

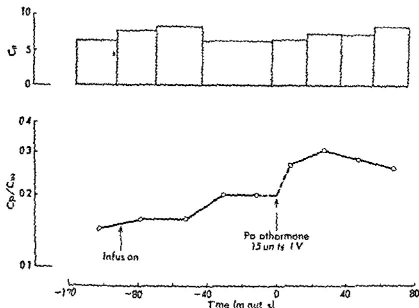


Fig 214 Effect of infusion of inulin in normal saline and of parathyroid extract on the clearance of phosphorus to the clearance of inulin ratio (C_P/C_{IN}) and clearance of inulin (C_{IN}) in an infant with tetany (age 1. days weight 3830 gm)

but do not answer many questions I think they do cast considerable doubt on the general conception that transitory hypoparathyroidism is the primary cause of elevation of serum phosphorus in young infants

Professor LEVINE (New York) If the kidney tubules do not make their decision to reabsorb phosphorus on the basis of the level of phosphorus in the serum, how is this to be reconciled with the observations on the relation of the increased phosphorus content of cow s milk to the appearance of tetany?

Professor BARNETT (New York) I think it is all compatible with the idea that a reduction in glomerular filtration rate is the primary cause of

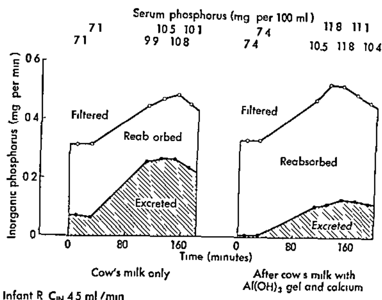


Fig 215 Effect of limitation of phosphorus intake on renal excretion of phosphorus before and after phosphorus ingestion Observations in a normal infant (age 30 days weight 2100 gm) on a high phosphorus intake and after five days of reduced phosphorus intake

elevation of serum phosphorus in the infant If the baby has a certain glomerular filtration rate and takes in more phosphorus from the diet he may not be able to excrete it and therefore the serum phosphorus rises The *inability of such an infant to reabsorb less phosphorus may mean that the* infant is unable to develop the so-called compensatory hyperparathyroidism which develops in renal disease It may be that the inadequacy of the new born infant is his failure to respond to an elevated level of serum phosphorus

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Professor WALLGREN (Stockholm) As there are no more questions we can regard this session as closed and since it is the last of the seminar I want to say a few words

It has been a real pleasure and an honor for me to be your chairman It *has not been the difficult task that I originally imagined it might be*—thanks to you You have never tried to impose a controversial opinion by raising your voices but rather by presenting evidence in the form of scientific data The only one who has raised his voice has been I believe myself when I had to shout to you to talk into the microphone I hope you will forgive me for that

The subject chosen by the World Health Organization—metabolism in

infants—has necessitated a close collaboration with specialties other than pediatrics and we have had outstanding representatives of chemistry bio chemistry physical chemistry nuclear physics and physiology They have contributed very largely to the increased knowledge that we have gotten every one of us at this seminar The characteristic feature of the seminar has been giving and receiving For us Scandinavians I can say that we have acquired much knowledge from you and I hope that even you Americans of the visiting team have gotten some new viewpoints and have been acquainted with somewhat different conceptions about many of the subjects we have discussed during this fortnight

I want to extend to all those who have presented these excellent introductory talks and to those who have participated in the panels and given us valuable discussion our sincerest thanks

Professor LEVINE (New York) I should like to express the profound appreciation of the visiting team not only for the way the group has been entertained socially but far more for the intellectual contributions which each member of the visiting team has I am sure received from the Scandinavians We came here with many qualms and much hesitation because we knew of the tremendous scientific importance of the fundamental contributions made by scientists from Denmark Finland Norway and Sweden All of the recorded information has been far exceeded by the fine performance of the Scandinavians The American team has received as much if not more than it has contributed As for me personally it was a rare privilege to be able to participate in seminars with such men as the three Nobel prize winners Professors Dam Hevesy and Tiselius and with the others who perhaps are as much deserving of this Swedish honor Again may I express my appreciation as the representative of the visiting team for the kindness we have been shown